Università degli Studi di Cagliari

PHD DEGREE

in Neuroscience

Cycle XXXIII

TITLE OF THE PHD THESIS

SEMIAUTOMATIC ANALYSIS OF SLEEP MICROSTRUCTURE PARAMETERS: AROUSAL, CYCLIC ALTERNATING PATTERN AND REM MUSCLE ATONIA.

Scientific Disciplinary Sector

06 Medical Sciences (MED/26 Neurology)

PhD Student:	Dr. Rosamaria Lecca
Supervisor	Prof. Monica M.F. Puligheddu
Coordinator	Prof. Paola Fadda

Final exam. Academic Year 2019 – 2020
Thesis defence: January 2021 Session

TABLE OF CONTENTS

Aknowledgements

Throughout the PhD years and the writing of this dissertation I have received a great deal of support worked with valuable people that accompanied me in this professional growth.

First, I would like to thank Prof. Monica Puligheddu for giving me the opportunity to carry out this project of thesis and for guiding me through all this years, allowing me to cultivate my passion for sleep medicine and grow professionally in both research and clinical practice.

Then I would like to thank Prof. Livia Fantini for welcoming me at Clermont-Ferrand in her research group. Working with her has been a stimulant experience and thought me to always be curious and accurate.

I also would like to thank Cristiano Rizzo and Nicola Rizzo for making possible my experience in Micromed® and guiding me through the six months in Mogliano Veneto.
I thank Cristiano, Tommaso Rizzo, Matteo Pilotto, Dmitri and Stefano Pellizzato for their work on the software CAP analyser, it's been a real pleasure to work with them.
I also thank all the other people in Micromed® for welcoming me and making me part of their fantastic group of work (and lunch!).
A very special thanks goes to Marina Ruotolo and her family for welcoming me in their house and making me always feel at home.

Thanks to all the colleagues of the Sleep Medicine Centre in Cagliari: Michela, Ludovica, Gioia and Patrizia for sharing with me their passion and knowledge in Sleep Medicine and Research.

Thanks to Alessandro for encouraging me day after day always being by my side.

Finally, I am grateful to all the patients participating to the study.

SUMMARY

This PhD project was developed in the context of the PON R&I 2014-2020 “Dottorati innovativi a caratterizzazione industriale” “PhD project with industrial characterization”, a particular PhD project that views the cooperation of the PhD student with an industrial company and a foreign University.

The thesis project is focused on systems of automatic analysis of sleep parameters.

This PhD thesis is composed of two main parts: the first part is focused on the process of creation of a software for the analysis of Cyclic Alternating Pattern (CAP) a particular parameter of sleep microstructure, and the second part is focused on the use of automatic analysis of muscle activity during sleep.

The company partner of this project is Micromed®, an international company for the manufacturing of hardware and software for neurophysiology based in Mogliano Veneto (TV). The PhD student spent six months at the company and worked side by side with the software programmers for the creation of a software that will allow the analysis of Cyclic Alternating Pattern with the Micromed® software for the Polysomnography (PSG) analysis.

CAP is defined as “periodic EEG activity of NREM sleep characterized by sequences of transient electrocortical events, that are distinct from the background electroencephalogram (EEG) activity and occurs at up to 1-minute intervals”.[1] CAP represents the microstructure of sleep, and its analysis gives fundamental information that are otherwise neglected with the analysis of sleep macrostructure (sleep staging) alone. CAP is considered a marker for the evaluation of sleep stability[2] and its oscillatory presence is fundamental preservation of sleep stability through the night and in response to arousal stimuli.[3] CAP rate, the percentage of CAP time during NREM sleep is an important parameter for the evaluation of sleep instability: an elevated CAP rate indicates an unstable sleep and is altered in different sleep disorders such as insomnia, disorders of arousal, epilepsy, sleep disorders breathing. On the other hand a very low CAP rate is found in all those sleep promoting condition such as Narcolepsy or in those

condition where there is loss of adaptive response such as in neurodegenerative disorders.[4]

The analysis of CAP is a very time consuming procedure and it is still used mainly for research purpose rather than in the clinical practice.

The development of a software for the analysis of CAP was the main focus of the work in collaboration with Micromed®. During the months spent at Micromed® facility the PhD student worked with the software programmers and engineers for the creation and validation of the software, individuating all the clinical parameters from guidelines[1] and verifying their correct application and the validity of the results. In the first part of this thesis all the creation process is described in detail.

The second part of this thesis is focused on the automatic analysis of muscle EMG tone during both REM and NREM sleep.

Muscle tone during sleep gradually diminishes throughout the different sleep stages reaching its minimum with REM muscle atonia.[5] Evaluation of muscle tone during REM sleep is fundamental for the diagnosis of REM sleep Behavior Disorder (RBD) in which there is a sustained muscle activity during REM associated to dream enacting behavior.[6]

Muscle activity is measured in polysomnography (PSG) through the recording of different EMG channels: chin, anterior tibialis muscle and flexor digitorum superficialis (FDS). The activity of those muscles is evaluated almost exclusively during REM sleep: the most used are manual methods such as the SINBAR[7] method and the Lapierre and Montplaisir[8] method, that require high expertise and are highly time consuming.

An automatic method developed in Italy (R. Ferri and co.) is now validated for the evaluation of loss of REM atonia in RBD: the calculation of REM atonia index.[9]

Few studies evaluated muscle tone during NREM sleep, and little is known about the neurophysiological mechanism that control muscle tone during this sleep phase.

Manual methods would be too difficult to apply to the whole sleep recording; on the other hand, the method developed by R. Ferri other than REM sleep, performs also a complete analysis of muscle tone for all sleep stages.

RBD may be considered as a dissociation of sleep states, with an intrusion of elements of wakefulness into REM sleep.[10] RBD is strongly associated to neurodegenerative disorders, especially synucleinopathies such as Parkinson disease, Multiple System Atrophy (MSA) and dementia with Lewy bodies.[11] Differential diagnosis between Parkinson disease and Multiple System Atrophy may be challenging. MSA patients have a more severe loss of atonia during REM sleep compared to Parkinson's with RBD. The research study was carried out during the six months at the foreign University in Clermont Ferrand (France) starting from the fortuitous observation of a prominent facial activity, we decided to evaluate the facial activity recorded in vPSG in patients with PD, MSA and controls and to evaluate the muscle tone in both REM and NREM sleep using the automatic method for the calculation of atonia index.

This is the first time that this automatic method is used extensively for the evaluation of muscle tone during NREM sleep.

Results of our study showed that MSA have a more sustained muscle tone compared to healthy controls in all sleep stages and compared to PD in all NREM stages. Moreover a particular facial expression was noted to be significantly more frequent in MSA compared to PD. This results may help the differential diagnosis between PD and MSA. Moreover the analysis of muscle tone during NREM sleep may open to different perspectives for the understanding of REM behaviour disorder and the mechanism underlying the control of muscle tone in NREM sleep.

The research work carried out in this thesis underlines the importance of the use of automatic or computer assisted methods for the analysis of different parameters of polysomnographic exams. Their use would allow to introduce in clinical practice and in research those analysis such as CAP analysis an analysis of muscle tone during both NREM and REM sleep that would be too time consuming to perform with visual and manual methods.

ABBREVIATIONS

AASM : American Academy of sleep Medicine

AI : Atonia index

CAP: Cyclic alternating pattern

DOA: Disorders of Arousal

ED: epileptiform discharges

EDF: European Data Format

EEG: electroencephalogram

EMG: electromyography

EOG: electrooculogram

MSA: Multiple System Atrophy

N1, N2, N3: stages of NREM sleep

NREM: Non Rapid eye movement sleep stage

NFLE: Nocturnal Frontal Lobe epilepsy

OSA: obstructive sleep apnoea

PD: Parkinson's disease

PSG: Polysomnography

RAI: REM atonia index

RBD: Rem Behavior Disorder

REM: Rapid eye Movement sleep stage

RSWA: REM sleep without atonia

SHE: Sleep Hypermotor Epilepsy

SWS: slow wave sleep

vPSG: videoPSG

PART 1 : DEVELOPMENT OF THE SOFTWARE FOR THE ANALYSIS OF CICLIC ALTERNATING PATTERN

Cyclic Alternating Pattern(CAP)

CAP: definition and scoring rules

Polysomnography (PSG) is the gold standard for the study of sleep and it is fundamental for the diagnosis and management of many sleep disorders and conditions.

Sleep is a dynamic process, based on physiological parameters two separate state have been identified within sleep: REM (rapid eye movement) sleep and NREM (Non REM) sleep.

Sleep staging is the process of definition of the sleep stages through the visual analysis of the different elements recorded with PSG. Sleep staging follows the rules written by American Academy of Sleep Medicine (AASM) in its manual:[12] sleep is analysed in 30 second epochs and the definition of the different sleep stages (N1,N2,N3, REM), leads to the designing of the hypnogram, which represents the sleep macrostructure.

The analysis of sleep macrostructure alone overlooks all the brief and transient events that occur during the 30-s epochs that represent an interruption of sleep continuity even if they do not lead to a stage shift.

The presence of these phasic events superimposed to the background activity is what in literature is defined sleep microstructure and their significance is believed to be the expression of the continuous balance between arousal and sleep maintenance forces.[13]

The first attempt to overcome this lack of information was made with the definition of arousal: an abrupt shift of EEG frequency that last 3-15 seconds. Nevertheless, even this definition provides a limited frame of the complex phenomenon of sleep microstructure, neglecting some transient phenomena such as K complexes, delta bursts or slow wave oscillations.

Finally, Cyclic Alternating Pattern (CAP), was described in 1985 by Parma research group: it is a periodic EEG activity occurring during NREM Sleep[14] and it represents an structured and recognized method that allows to frame the analysis of sleep microstructure.[3]

CAP is defined as "a periodic EEG activity of NREM sleep characterized by sequences of transient electrocortical events, that are distinct from the background electroencephalogram (EEG) activity and reoccur at up to 1-minute intervals".[1]

The paper "Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep"[1] describes in detail all the aspects of this phenomenon and is the guideline for its scoring, all the features and scoring criteria described below are contained in this reference.

The periodic activity of CAP is characterized of the alternance abrupt frequency and amplitude variations (A-Phase) followed by the return to the background activity (B-Phases).

Both A phase and B phase have a duration of 2-60 seconds, B phase can be scored only when another A phase occur in another 60 seconds following the first A-phase.

An A-phase followed by a B-Phase compose a CAP cycle.

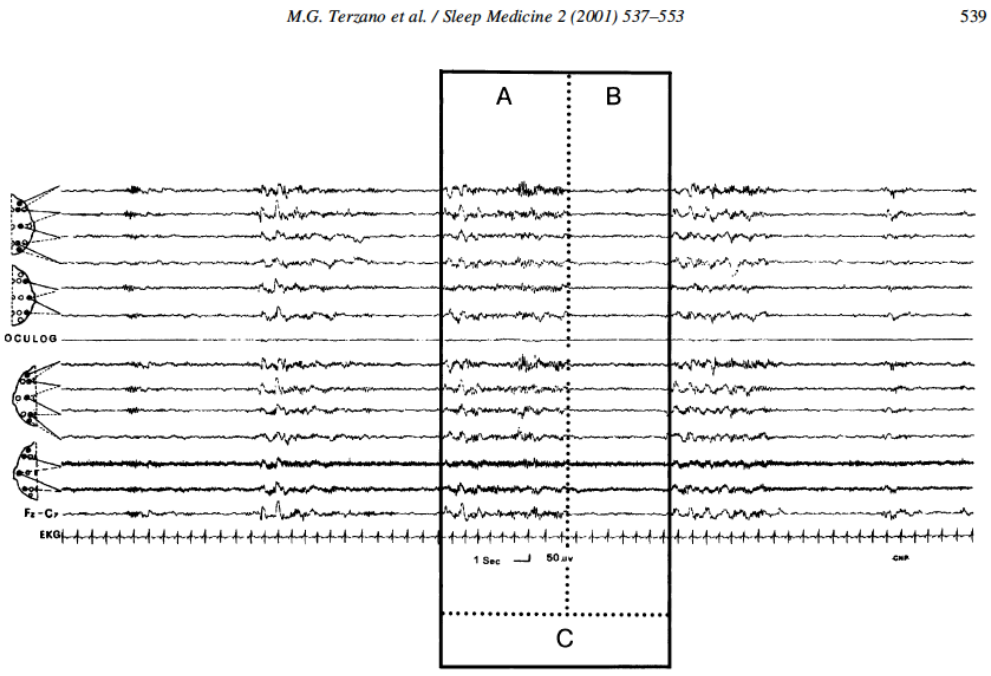
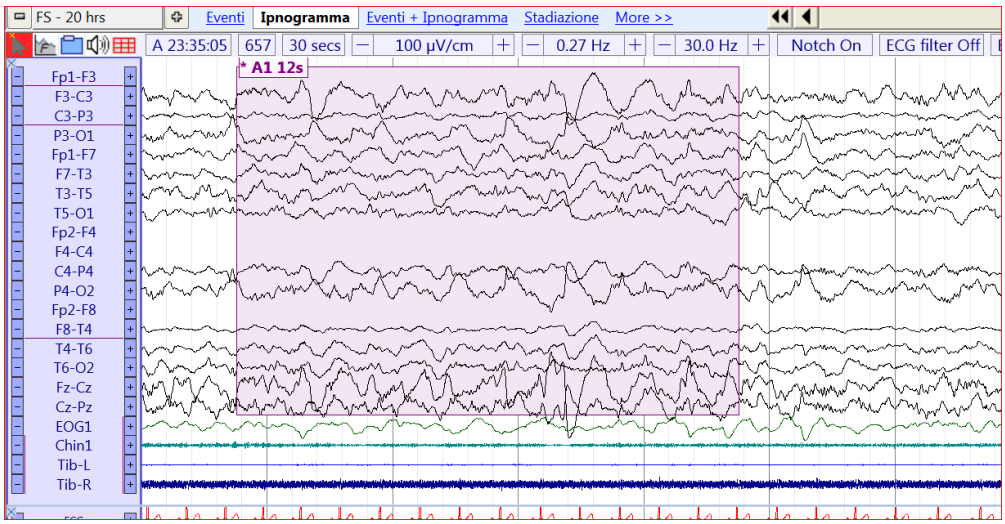


Figure 1 CAP Cycle. Terzano et al. Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern) in human sleep

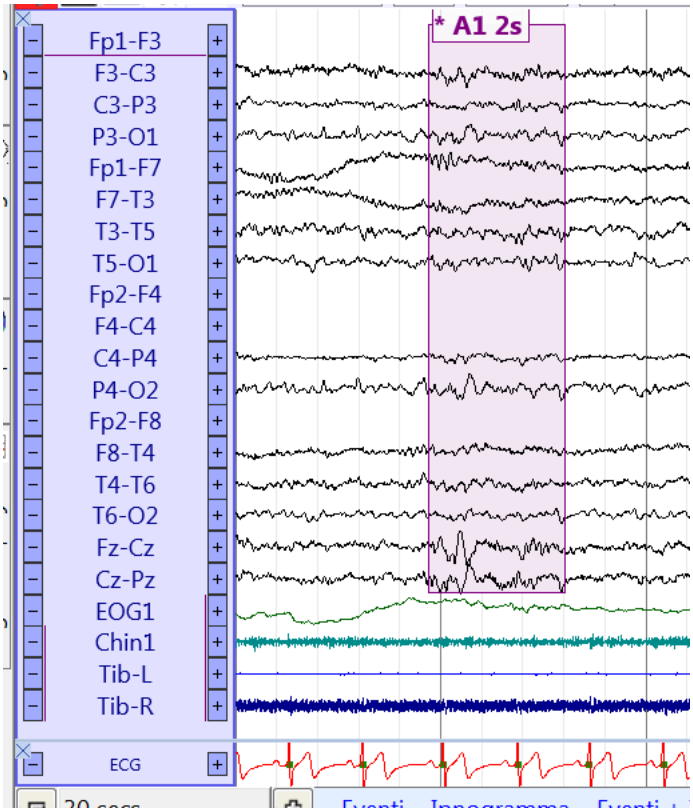
An A-phase should be visible in all the EEG leads, therefore the standard bipolar montage (Fp1-F3, F3-C3, C3-P3, P3-O1 or Fp2-F4, F4-C4, C4-P4, P4-O2) used for recording of polysomnography (PSG) is sufficient for the detection of this phenomenon. Monopolar derivations can be used, electrooculogram (EOG), chin and tibial muscles electromyography (EMG) are also required as a part of a PSG. Additional channel such as nasal flow or thermistor for the respiration, electrocardiogram and pulsometer allow the identification of related autonomic changes or respiratory event related to the A-phases.

The A-phase is identified as a transient event that clearly stands out from background EEG activity, bringing an abrupt and brief shift of frequency and amplitude.
A-phases can be composed of rhythms of different frequency and amplitude, that may be frequently mixed during the same event. There are several EEG elements that can be part of the CAP A-Phase: Delta bursts, Vertex sharp transients, K-complex followed or not by spindles, polyphasic bursts, K-alpha, Intermittent alpha, EEG arousal.
Here is a detailed description of A phases Events:

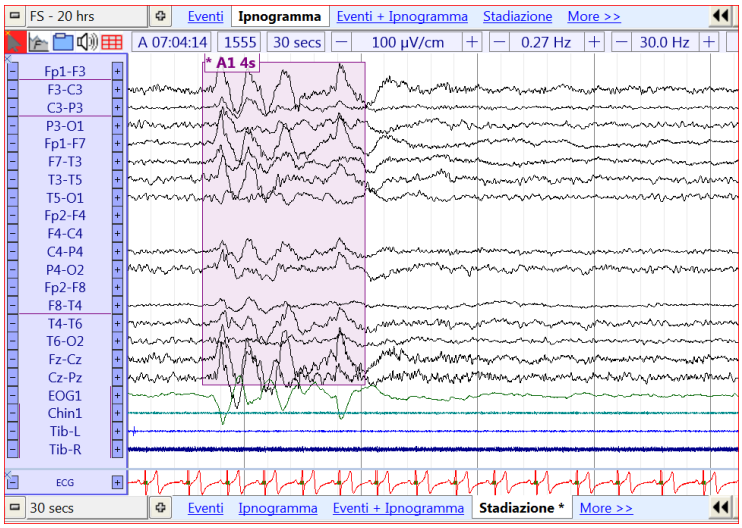
- Delta bursts: a sequence of at least two waves in the frequency band of delta EEG (0,5-4Hz) that emerges from background activity with an amplitude that is 1/3 higher. Their presence is characteristically seen in advanced N2 and in N3 stage.



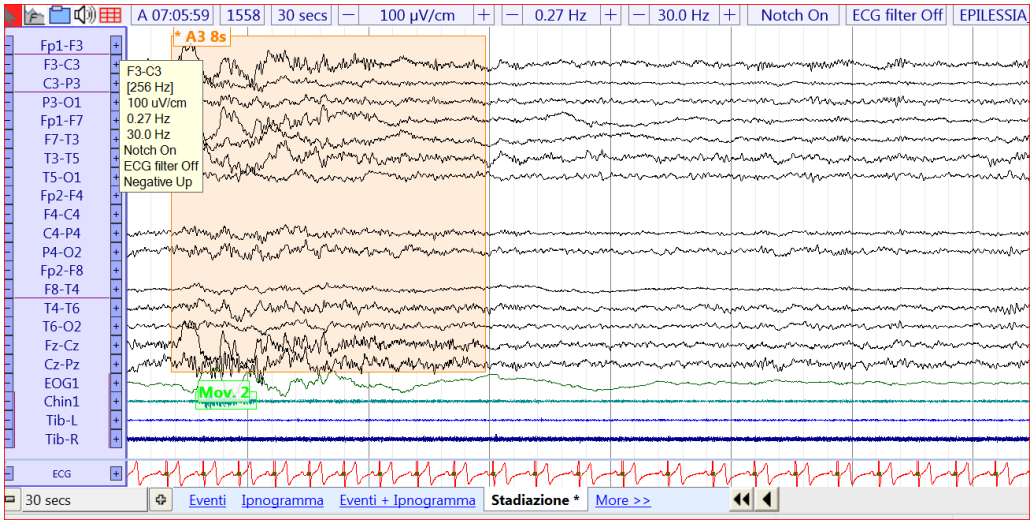
- Vertex sharp transient: transient EEG wave with a duration of 50-200 ms and variable amplitude (up to 250 μV).



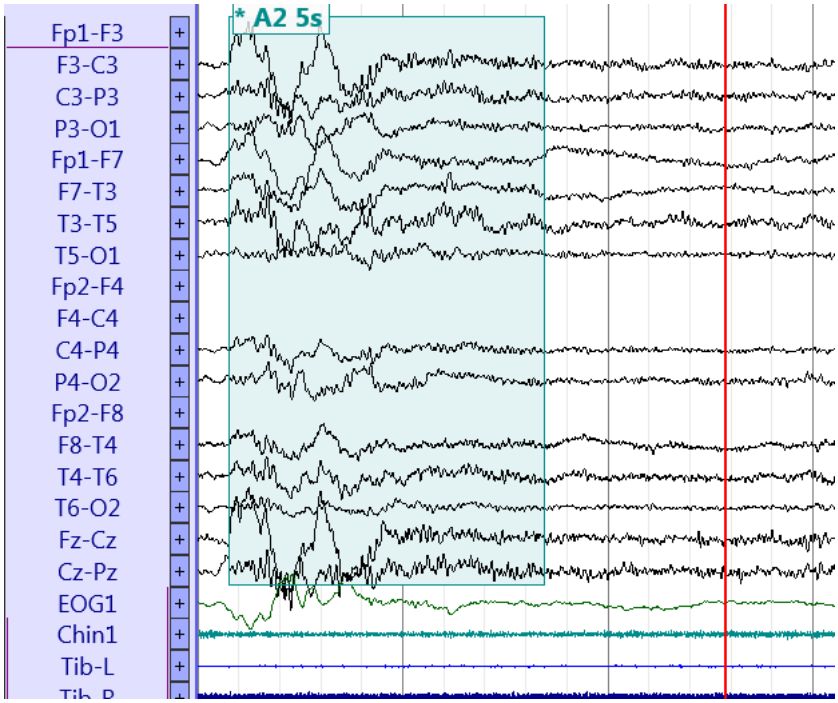
- Sequences of K-complexes: K-complex is a well-defined figure of sleep and its presence is fundamental for the scoring of N2 sleep, but it can occur also during N3. A K-complex is characterized by an initial negative sharp wave followed by a lower positive component with a total duration >= 0,5 seconds.



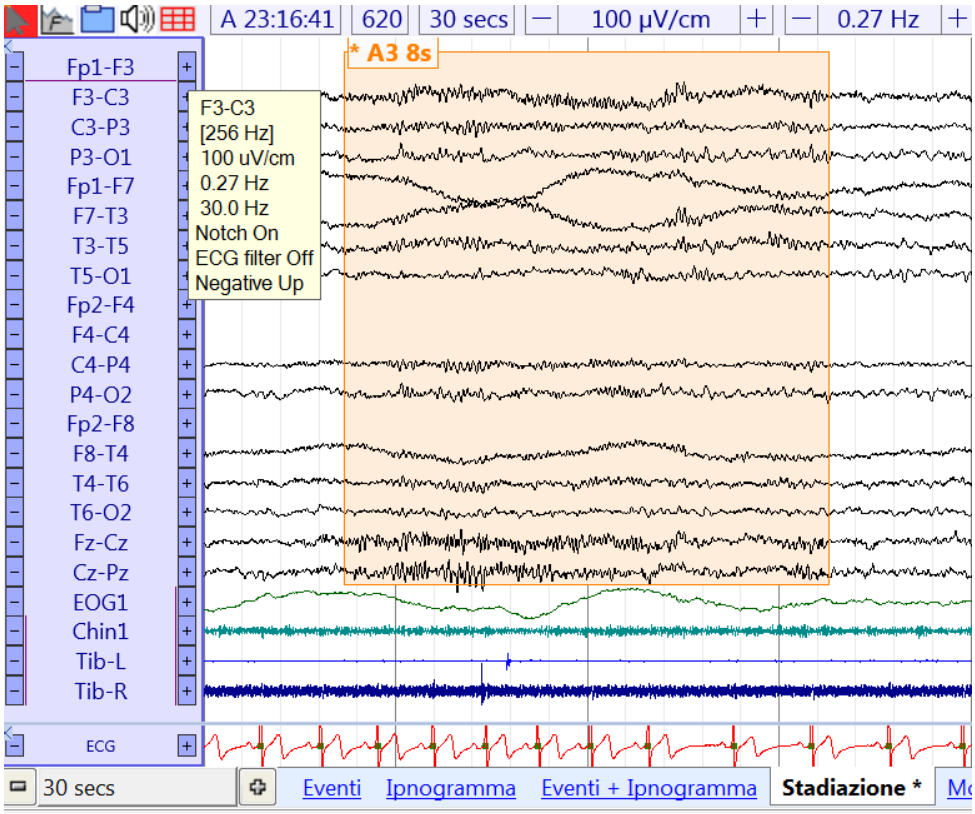
- Polyphasic bursts: a cluster of high voltage delta waves mixed with theta, alpha or beta rhythms. They usually occur before the transition from N2 to REM.



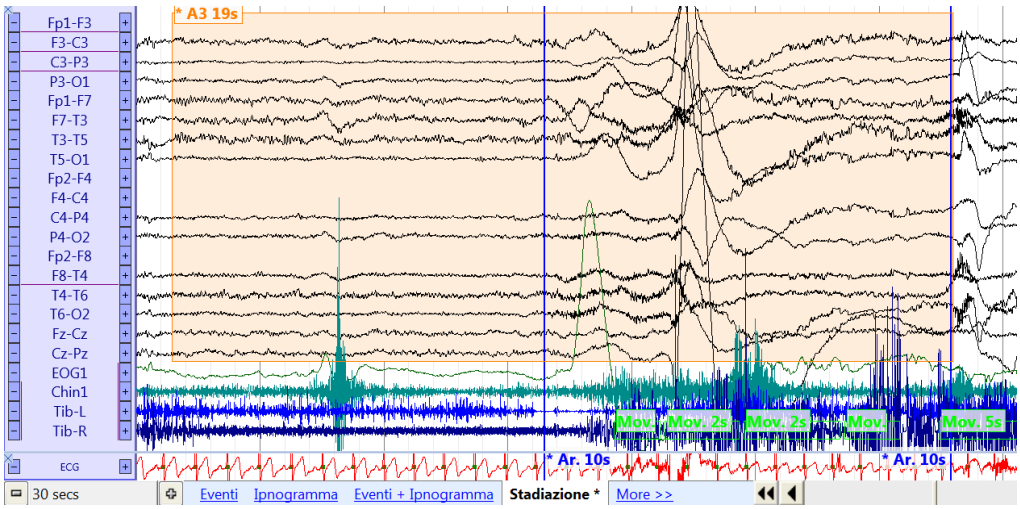
- K-alpha: a K-complex followed by burst of alpha activity.



- Intermittent Alpha: after falling asleep, the normal alpha activity (8-13Hz) that is seen in quiet wake with eyes closed spreads from the occipital region to a more anterior representation. Little sequences of alpha are seen in stage N1 and disappear when sleep progresses.

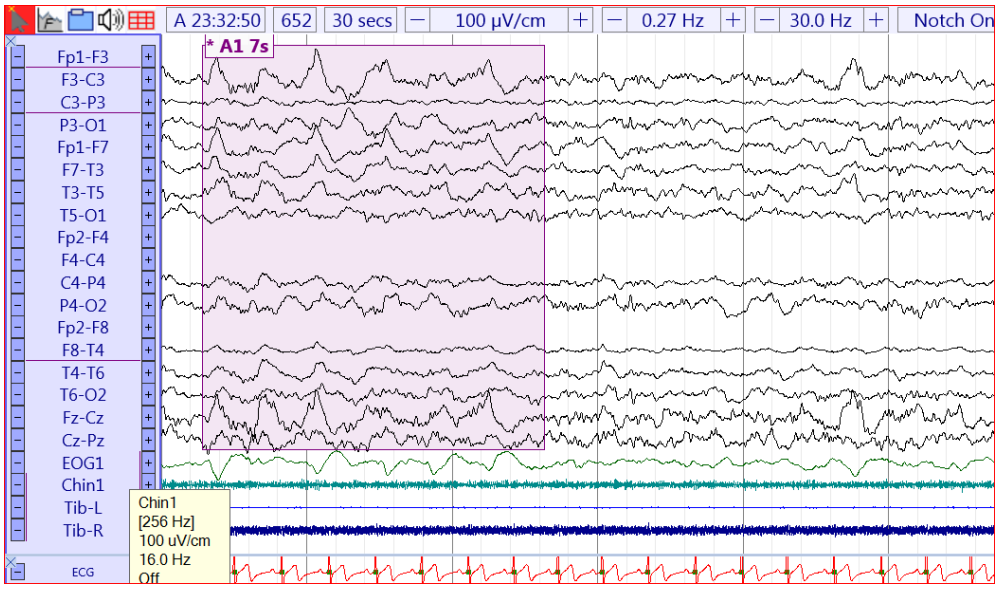


- EEG arousal: ASDA arousal are defined as a sudden change in rhythm toward a faster rhythms than background.

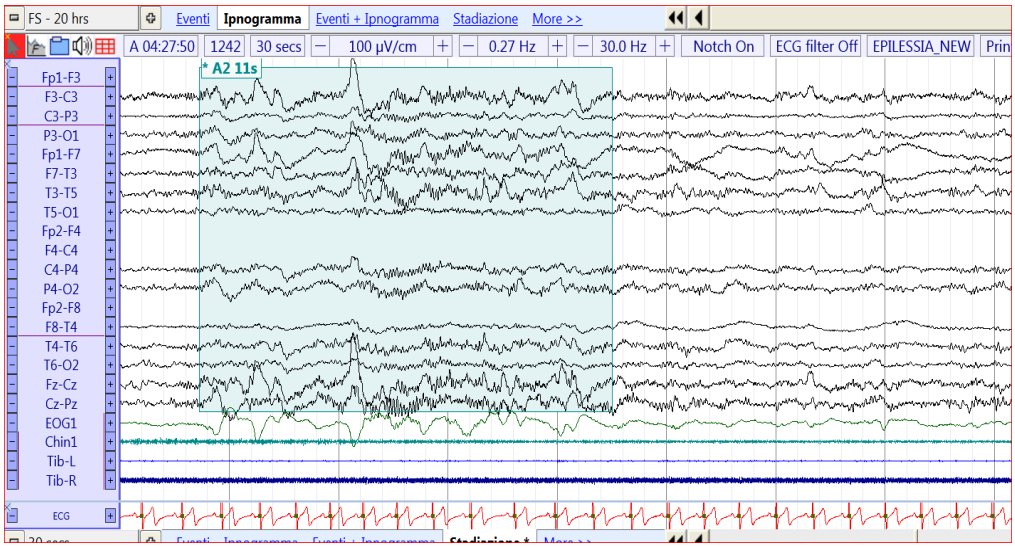


The A-phase is classified into three subtypes accordingly to its degree of desynchronization:

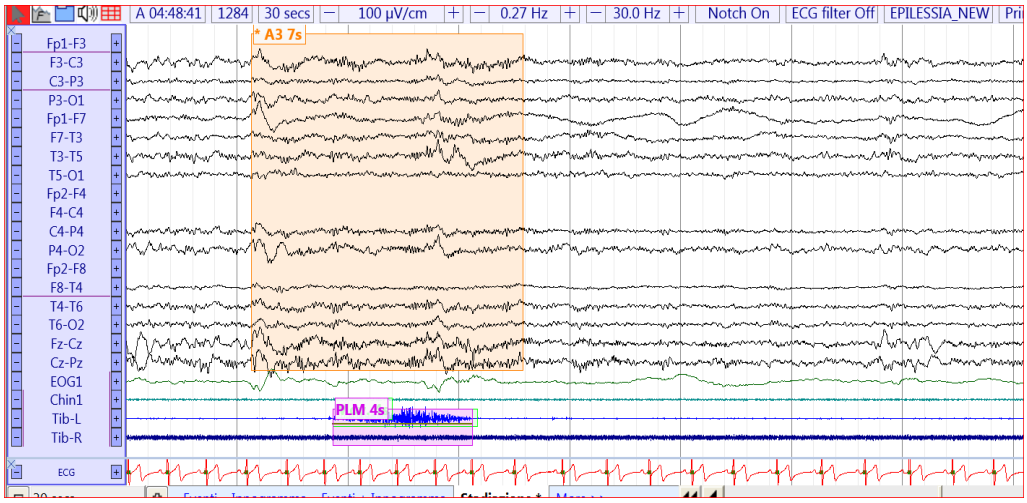
- A1: EEG activity is predominantly synchronous, if desynchronization is present it does not exceed 20% of the duration of the episode. Alpha rhythm, delta bursts, K-complex and vertex sharp transient are more commonly observed in this subtype.



- A2: EEG activity is a mixture of rapid and slow rhythm, but desynchronization occupies less than 50% of the A-phase.



- A3: EEG activity is desynchronized, with rapid and low voltage rhythms, K-Alpha, EEG arousal and polyphasic bursts are the figures predominantly observed in this subtype. If a movement artifact occurs within a CAP sequence, it must be scored as an A3 phase.



Different A phases can be found in the same CAP sequence.

A CAP sequence is made of a succession of two or more CAP cycles. All CAP sequences begin with an A-phase and terminate with a B-phase, consequently three or more A-phases are necessary in order to identify a CAP sequence.

There are no upper limits on duration and on number of cycles contained in a CAP sequence. Within NREM sleep a CAP sequence is not interrupted if a change shift in sleep stages occurs, it is only interrupted by the onset of wake or REM sleep.

Under normal circumstances CAP does not occur during REM sleep, however some pathological conditions (e.g. sleep apnoea) can trigger the presence of A-phases repeating at intervals <60 seconds, creating a CAP sequence during REM. Those sequences are not normally included into the parameters computed for CAP reporting.

The absence of CAP for more than 60 seconds (when the interval between two consecutive A-phases exceeds 60 seconds) is scored as non-CAP sleep.

An isolated A-phase and the A-phase that closes the CAP sequence are counted as non-CAP.

CAP sequences and non-CAP periods can be considered as the phasic and tonic components of NREM sleep and they alternate throughout all its evolution during the night.[15]

CAP: neurophysiological and clinical significance

In order to understand the significance of CAP, the concept of arousal must be kept in mind: Arousal is defined by American Academy of Sleep Medicine (AASM), rules for scoring as an abrupt shift of EEG frequency (including alpha, theta and frequencies >16 Hz) that lasts for at least 3 seconds after 10 seconds of a stable sleep EEG.[16] In association to the EEG arousal a behavioral or an autonomic change can be observed. Scoring of arousals is a fundamental part of the scoring and reporting of a PSG and Arousal index is a marker of sleep quality. Nevertheless, as the scoring of macrostructure of sleep, ASDA Arousal index provides a statical and rigid vision of the phenomenon.[17]

CAP was initially described as an activity occurring during situation of impaired vigilance (stupor or coma) made by the alternance of periods of high voltage slow wave (A-phase) separated by a low voltage activity (B-phase). A-phases in coma are associated to periods of hyperventilation, greater muscle activity and increased heart rate, representing a sort of "activation" or arousal from the state.[18]

CAP in coma and stupor represents a marker of cerebral activation, its absence coincides with an arousal stability whether its presence is responsive to different external inputs. The presence of the sudden the appearance of slow and high voltage activity in response to an external stimulus occurring during coma is observed also during NREM sleep.[2]

If an stimulus is delivered during non-CAP sleep, there are hypersynchronous and brief responses that hare subject to progressive habituation, if the stimulus intensifies (e.g. an increasing acoustic stimulus) a CAP sequence is immediately evoked.[19]

If the stimulus is delivered during the B-phase of a CAP sequence there is an immediate shift to an A-phase, that is stereotyped and lacks habituation, even for minimum intensity stimuli.

The CAP sequence evoked by an external stimulus may lead to a lightening of sleep depth or continue as an oscillation before the complete recovery of NCAP sleep .

A-Phase of CAP can be considered as a sort of preattentive response to sensory stimulus: providing a microstructural response the brain creates a flexible adaptation that preserves the macrostructure from external perturbation.[20]

The stability of the sleep macrostructure is protected by the instability of sleep microstructure, but it is also seen that with an increase of the CAP rate the sleep becomes shallower and more fragmented. Instability of CAP act as both a protective factor and a preparatory tool for macrostructural alterations.[3]

CAP may be compared to a "director" of sleep, that coordinates responses in different regions of the brain and accompanies the evolution of sleep through stage transition, awakenings and arousals.[21]

Sleep is regulated by a complex interaction of different systems: the circadian model (process C) with multiple oscillators that regulates the sleep wake rhythm (body temperature, melatonin) the homeostatic process (process S), based on the rise in sleep pressure during the day and its decay during the night of sleep; and the ultradian model, the physiological alternation of the aminergic REM off neurons and the cholinergic REM-off neurons.[22]

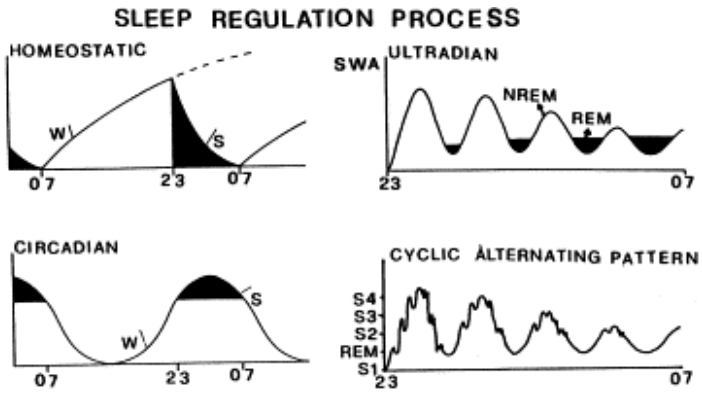


Figure 2 Representation of models of sleep regulation: Homeostatic process (S), Circadian rhythm (C), ultradian regulation and alternance of NREM and REM and Cyclic Alternating Pattern. Parrino et al 2010 Cyclic alternating pattern (CAP) and epilepsy during sleep: how a physiological rhythm modulates a pathological event

As it can be seen from an hypnogram of a normal night, a sleep cycle can be schematically represented beginning with a descending branch, expression of the deepening from wakefulness trough the different stages of NREM sleep; a steady phase of stable slow wave sleep (SWS) and an ascending branch preceding the onset of REM sleep, with a passage from a state of EEG synchrony to a EEG desynchronization.

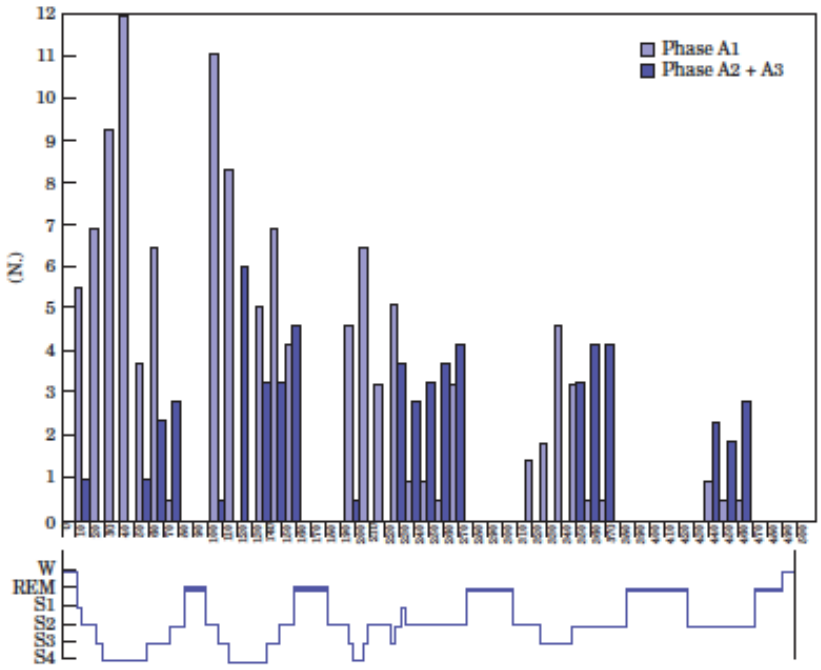


Figure 3 Distribution of A-Phases during the night (Parrino et al, 2000, Origin and significance of CAP)

CAP A1 phases are more common during the descending branch of the sleep cycle, CAP is less represented during the steady phase and during the ascending branch there is a majority of A2-A3 phases.[23]

The distribution of A1 in the descending branches of the hypnogram, during the building up of the SWS sleep and the wider distribution of A2 and A3 phases before the onset of REM sleep suggests that the expression of CAP may be influenced by the same neurophysiological processes that regulates the alternance of REM and NREM: A1 phases may depend on the activity of the aminergic REM off neurons and A2-A3 from the cholinergic REM- off neurons.[23]

A1 phases show an exponential decline during the progression of the sleep cycles throughout the night, reflecting the decay of the homeostatic process, A2 and A3 phases distribution was not influenced by the distribution of the sleep cycles, occurring particularly before the onset of REM sleep, and this strengthens the hypothesis of their dependence from REM on neurons.[24]

As previously stated, CAP occurs physiologically during NREM sleep or can be evoked by external or internal stimuli such as noise or a breathing event like an apnoea.

Every transient phenomenon that occurs during CAP has a different origin in the brain: Vertex sharp transient may be considered as evoked potential, they are similar to acoustic evoked responses in wake and they are thought to have an excitatory significance; delta bursts are of cortical origin and have a thalamic influence and modulation; K complexes are a cortical phenomenon that can be associated to an increase of sympathetic activity.[3]

CAP rate is percentage of CAP time (the sum of the duration of all the CAP sequences) in non-REM sleep and it can be considered as a marker of sleep instability.

CAP rate is increased in several sleep disturbances: insomnia, obstructive sleep apnoea (OSA), sleep-related epilepsy, disorders of arousal (DOA), periodic limb movements (PLM).[4] On the other hand a very low CAP rate is found in all those sleep promoting condition such as Narcolepsy or in those condition where there is loss of adaptive response such as in neurodegenerative disorders.[4]

CAP rate in healthy subjects varies throughout lifetime, with a U-shape profile: its minimum is found in young adults (31,9%) and its maximum in the elderly (55%), with intermediate values in teenager (43%) and middle-aged people (37,5%). Elderly groups have longer sequences and an higher occurrence of A2 and A3-phases, whether A1-phases are more represented in younger subjects.[25] In pre-school age children CAP rate is 25%[26], and in school-aged children, in school age children in 33%, with the lower level of A2 and A3 registered across lifespan.[27]

CAP rate in adults has a low night to night intra-individual variability.[28]

Applications of CAP

Although CAP provides fundamental information about sleep and its use would be desirable for the clinical evaluation of different pathologies such as insomnia, disorders of arousal, epilepsy, its use in clinical practice is not routinely performed and CAP analysis is not integrated in most of the PSG reading software.

CAP scoring is a very time-consuming process and require highly trained personnel to perform the analysis.

Even if performed by experts the analysis of CAP presents with an interscorer variability: inter scorer agreement was evaluated in only three studies: in one study it was assessed to be above 70%[29] and the other study found a Kendall W coefficient of concordance of 0,9 for Total CAP time and CAP rate.[30] The most recent study showed a pairwise inter-scorer agreement between 55,5 and 85% with a global average of 69%.[31]

Moreover the inter-rater reliability of the CAP is also subordinated to the presence of an inter-rater variability for scoring of sleep stages which is not 100% and is estimated to be around 80%.[32]

All those studies are performed with only a few number of scorers and there is not a comparison with different level of expertise, a more extensive evaluation is needed in order to assess the agreement score also for a better evaluation of eventual automatic-reading systems.

Some attempts of the creation of an automatic detection of A-phases have been made with the use of machine learning methods and spectral analysis, results are promising[31,33] but yet reliability of those algorithms has to be improved.[34]

CAP and Epilepsy

Seizure and interictal epileptiform discharges (ED) during sleep are mostly entirely seen during NREM sleep, while they are extremely rare during REM sleep.[35]

The biological mechanism of this interaction is not fully known: SWS and its oscillatory elements such as spindle K-complex, CAP and arousal sleep seems to “unmask” the

epileptiform networks and this complex interaction may play a role in neuronal plasticity and cognitive functions.[36] Interaction between sleep and seizures is reciprocal: the presence of ED and seizures causes a disruption in sleep and alteration of sleep stability and architecture.[37]

Considered this phenomenon, it is straightforward that CAP plays an important role in seizure occurrence and ED and once again different studies performed on epileptic patients compared to healthy controls showed that whether macrostructure does not provide any additional information on sleep of epileptic patients, the microstructure of sleep is altered.

The first demonstration that ID and epileptic seizures during sleep occurs mostly during A-phases of CAP, with a strong inhibition during B-Phases was made in 1992 on patients with juvenile myoclonic epilepsy[38] and this finding is confirmed in later studies on primary generalized epilepsy and lesional epilepsy with fronto-temporal focus.[39]

The syndrome previously called Nocturnal Frontal Lobe Epilepsy (NFLE) has been redefined in its definition in the last few years and renamed in Sleep Hypermotor Epilepsy (SHE), because there was major consensus on the non-exclusive origin from frontal lobe.[40]

This syndrome is characterized by the occurrence of brief (<2 minutes) seizures with stereotyped motor patterns within individuals and abrupt onset and offset, mostly "hypermotor" that occur predominantly during sleep, even if rarely seizures during wakefulness may occur.[41]

Although patients with SHE complain of poor sleep quality, daytime tiredness, there are no differences in macrostructure with healthy controls, but the analysis of microstructure with CAP scoring reveals an increased CAP rate indicating a higher sleep instability.[42]

Another study demonstrated that patients with SHE have an increased CAP time, CAP rate CAP cycles, and mean duration of a CAP sequence compared to control group, all seizures recorded occurred in NREM sleep and 90% of them occurred during a CAP sequence, in association with an A-phase.[43]

The occurrence of ED and minor motor events is strongly associated with CAP sleep in SHE.[44]

Interictal ED and CAP sleep may have a reciprocal triggering role in the occurrence of sleep related seizures: ED facilitates the occurrence of micro-arousals and increase sleep instability. This instability increases the occurrence of minor motor events and facilitate the occurrence of seizures, that at their turn sustain sleep instability.[45]

CAP and disorders of arousal

Disorders of (DOA) are classified in International Classification of Sleep Disorders 3rd edition (ICSD-3)[46] as NREM sleep parasomnia, and are characterized by a broad spectrum of manifestations varying from confusional arousal, motor manifestation and complex behaviours such as sleepwalking or night terrors.

NREM parasomnia are more frequent during childhood and they tend to resolve through puberty, but they can persist or emerge during adulthood with a 2-4% prevalence of adults and 17-27% of cases as adult onset.[47]

DOA episodes might be associated with vegetative symptoms, automatic behaviours, misperception and metal confusion and show a fluctuating course, alternating periods of high frequency and intensity of episodes followed by free intervals.[48]

Differential diagnosis between DOA and nocturnal epilepsy may be challenging: although semeiology largely differs in the two entities, the presence of brief episodes of seizures with paroxysmal arousal can be very similar to the simple arousal movements typical of DOA; in the recording of brief episodes only in v-PSG is not sufficient for a reliable diagnosis.[41]

Moreover an higher occurrence of minor motor movements during sleep in SHE, may be a sign of increased sleep instability: interictal epileptic discharges may increase microarousal and CAP rate creating a vicious circle that facilitates the occurrence of other motor events of both epileptic and non-epileptic nature.[45]

In DOA sleep macrostructural parameters do not differ from those of healthy controls, on the other hand microstructural analysis shows an increased CAP rate and an increased number of CAP cycles.[49]

Instability of NREM sleep, in terms of alteration of microstructure with increased CAP rate, seems to be an intrinsic condition of DOA, this alteration persist even in those nights that are free from motor episodes.[50]

Alteration of CAP are observed moreover during SWS in children with night terrors, with frequent inclusion of B-phases that interrupt continuity of sleep and an increase of A1 index.[51]

A disturbance in the continuity of SWS is seen also in patients with sleepwalking[52]: the presence of alteration of delta power in the first cycles of the night is associated to an increased A2 and A3 activity in patients with sleepwalking.[53]

Considering that analysis of microstructure alone does not provide important information in patients with DOA, analysis of CAP should be integrated in the evaluation of those patients.

Creation process and validation of "CAP Analyzer" Software

The software is the fruit of the collaboration of the PhD student with Micromed®, an Italian company based in Mogliano Veneto (TV) that is specialized in the creation and commercialization of devices and software for neurophysiology. Micromed® produces devices and software for the recording and analysis of Electroencephalogram, Polysomnography and Electromyography.

The PhD student spent six months in the company R&D (Research & Development) Department. During those months the PhD student had the chance to work side by side with the engineers and programmers that work on the development of the new software and hardware.

Not only it was a chance to develop the PhD project and participate to the creation of the software, but also a chance to know all the work behind the creation of the software that are currently used in clinical practice.

Software specifics

Micromed System Plus Evolution® is the software for the acquisition of EEG and PSG. This acquisition system is used worldwide for EEG long term telemetry (LTM) and sleep studies. Both LTM and PSG can be performed in laboratory with a video surveillance or as ambulatorial exams at home.

SystemPlus Evolution has now been updated with a new version Brain Quick®.

Analysis of PSG in Systemplus® and Brain Quick® is performed through another software integrated in the system: SleepRT™ a software for the sleep analysis of sleep created by OSG®, a software company owned by Micromed® group.

"CAP Analyzer" is a software written in C# for the semiautomatic analysis of CAP with SleepRT™.

With SleepRT™ the operator performs the sleep staging (N1,N2, N3, REM), as it is normally done in clinical practice.

All the A-events are manually detected by the operator.
After launching the analysis of PSG in order to obtain the normal PSG report, SleepRT™ creates an event file in which every event is noted with its time of beginning and end.
CAP analyser works reading this event file and creates a report of the CAP analysis.
How the software runs and the work done during the phases of its creation are described in in the next paragraphs.
CAP analyser is a software written in C# for the semiautomatic analysis of CAP with SleepRT™.

All the phases of the creation were the followed step by step by the software programmers in Micromed® and the PhD student.
The PhD student is a clinical researcher and expert in sleep medicine, during the first year of PhD program she was trained for the analysis of CAP that requires high expertise.
The PhD student supervised all the phases of creation of the software (individuation of correct parameters and requirements) and validated all the results that are described in the next paragraphs.

Phases of CAP Analyzer Software Creation

Detection of CAP Cycles

A pool of different PSG exams of healthy patients was manually analysed: and all the A-Phases were visually detected by the PhD student in order to create a starting point for the creation of the software.

The first step was to define all the parameters and requirements for the individuation of the CAP cycles, CAP sequences and CAP-non-CAP time.

All the specific parameters that were decided to be used met the criteria described in the “Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep”[1] paper, which is the guideline for the CAP scoring rules.

In its initial step the software analyses the event file created by SleepRT™.
The program reads the “.xml” input file that is composed of a single block called "BrainRT Results" which contains two large sub-blocks: the first contains the events defined by the user (CAP events), the second contains the specific events identified with type, subtype, validity, start and end.

The event file reports all event types: every event is codified with a number which is univocal for the different event.

```
        <Type>
                <Description>Evento definito dall'Utente</Description>
                <Label>UDE</Label>
                <Code>32768</Code>
        </Type>
        <SubType>
                <Description>luci accese</Description>
                <Label>luci accese</Label>
                <Code>32773</Code>
                <Colorref>-16738816</Colorref>
                <Guid>176264C3-067A-492C-8506-7E56494FF185</Guid>
        </SubType>
</EventDefinition>
<EventDefinition>
        <Type>
                <Description>Evento definito dall'Utente</Description>
                <Label>UDE</Label>
                <Code>32768</Code>
        </Type>
        <SubType>
                <Description>Fase A1 CAP</Description>
                <Label>A1</Label>
                <Code>32768</Code>
                <Colorref>-65536</Colorref>
                <Guid>BB25FBBD-A67C-4C7E-BE29-000FFC35FDBA</Guid>
        </SubType>
</EventDefinition>
<EventDefinition>
        <Type>
                <Description>Evento definito dall'Utente</Description>
                <Label>UDE</Label>
                <Code>32768</Code>
        </Type>
        <SubType>£
                <Description>Fase A2 CAP</Description>
                <Label>A2</Label>
                <Code>32769</Code>
                <Colorref>-16776961</Colorref>
                <Guid>5679D8B8-B887-4194-8F78-9EC6E662A060</Guid>
        </SubType>
</EventDefinition>
<EventDefinition>
        <Type>
                <Description>Evento definito dall'Utente</Description>
                <Label>UDE</Label>
                <Code>32768</Code>
```

Figure 4 Definition of events in the Event File

For every occurring throughout the recording event SleepRT™ software defines its beginning and its end as showed in figure:

```
        <Validated>false</Validated>
        <Start>2017-10-24T21:17:14.110000</Start>
        <End>2017-10-24T21:17:14.110000</End>
        <CH1/>
        <CH2/>
</Event>
<Event>
        <Type>128</Type>
        <SubType>301</SubType>
        <Validated>false</Validated>
        <Start>2017-10-24T21:41:50.000000</Start>
        <End>2017-10-24T21:45:20.000000</End>
        <CH1/>
        <CH2/>
</Event>
<Event>
        <Type>32768</Type>
        <SubType>32770</SubType>
        <Validated>true</Validated>
        <Start>2017-10-24T21:41:50.188206</Start>
        <End>2017-10-24T21:42:04.868256</End>
        <CH1/>
        <CH2/>
</Event>
<Event>
        <Type>32768</Type>
        <SubType>32770</SubType>
        <Validated>true</Validated>
        <Start>2017-10-24T21:42:50.075282</Start>
        <End>2017-10-24T21:43:03.870765</End>
        <CH1/>
```

Figure 5 Event definition by SleepRT™

CAP analyser works on the analysis of the event file reading all the events and their time of starting and end and their position throughout the recording time.

Every time interval of time detected by the analysis of the .xml is labelled accordingly to a different condition and this “labelling” is categorized in different ways during the analysis.

The first layer of the analysis is the detection of CAP cycles and non-CAP time.

The first condition that if an A-phase is separated by more than 2 seconds and less 60 seconds from another A-Phase then the software detects this separation time: if it fulfils those requirements then it scores the separation as a B-phase and the event of a A+B phase is detected as CAP Cycle.

Phase that does not belong to a cycle are listed as isolated A-phases and their interval time is scored as non-CAP time.

The first version of the software created a list of all CAP cycles with their beginning and end.

All the results were validated with a manual and visual check of the correspondence of the results obtained by the software with the PSG trace.

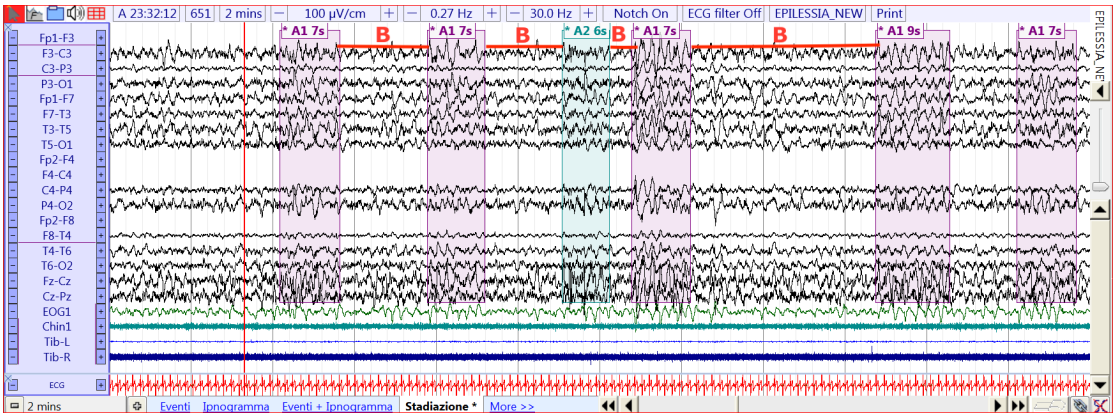
The program then creates a list of CAP cycles that will be used for the subsequent analysis of CAP sequences.

Detection of CAP sequences

The second phase of the creation of the software was the detection of CAP sequences.

A CAP sequence is composed by two or more CAP cycles. It must begin with an A-phase and ends with a B-phase. A single sequence may contain an indefinite number of cycles, and they can of all three types.

This is an example of a CAP sequence composed by 5 CAP cycles.



The program through the analysis of the list of cycles created in the first interaction detects the presence of the sequence: every CAP cycle separated by less than 60 seconds than another CAP cycle is listed into the specific sequence.

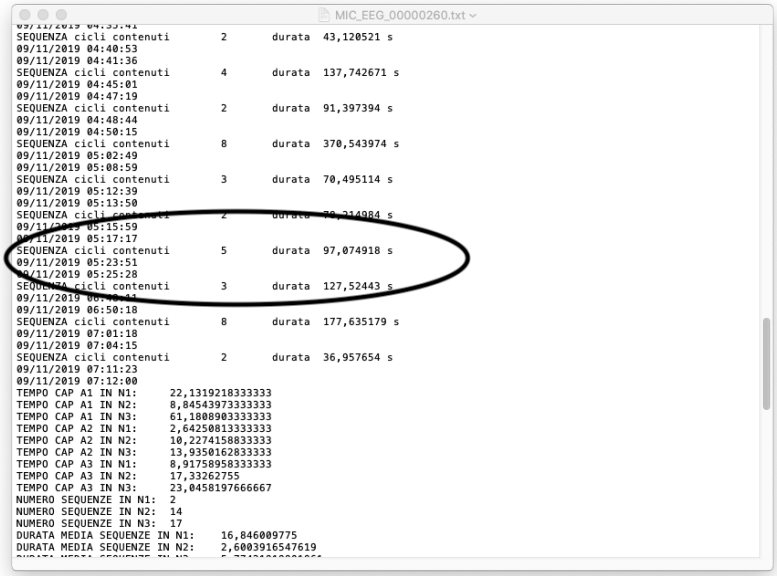
The sequence can have an indefinite length.

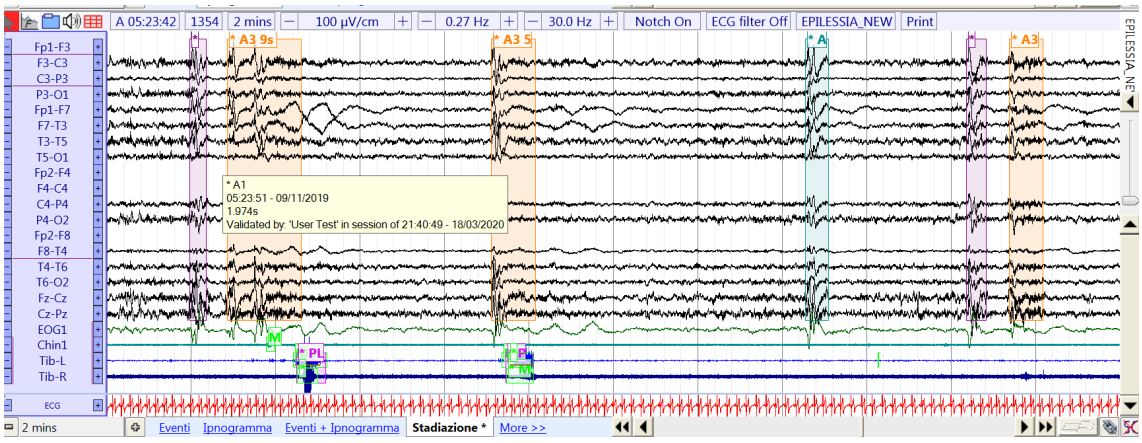
After the computing of this part, all the process went through manual validation.

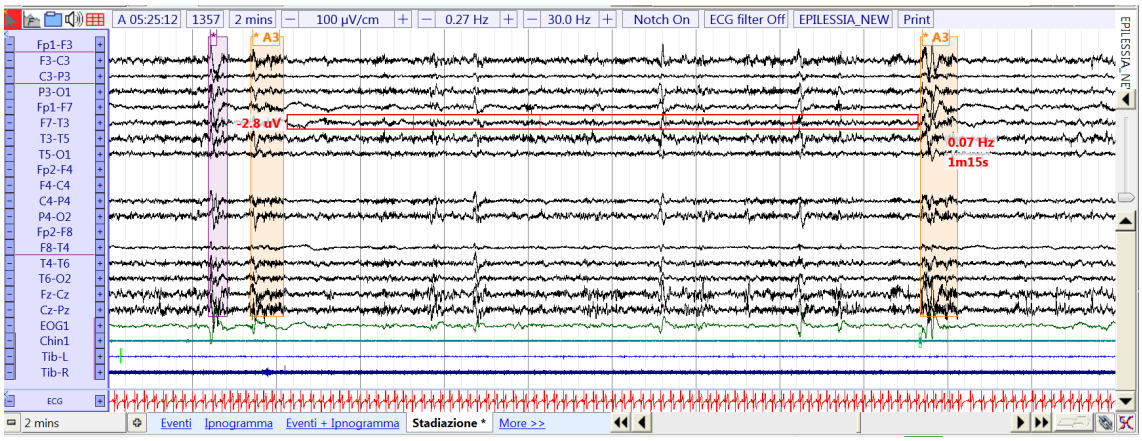
The program creates a list of sequences and the clinical expert validated the sequence.

This is an example of a sequence detected in a PSG that was analysed with the software CAP analyser.

The sequence starts at 05:23:51 and ends at 05:25:28 , which is the beginning of the first A3-phase that is shown in the second figure. The sequence contains 5 cycles.

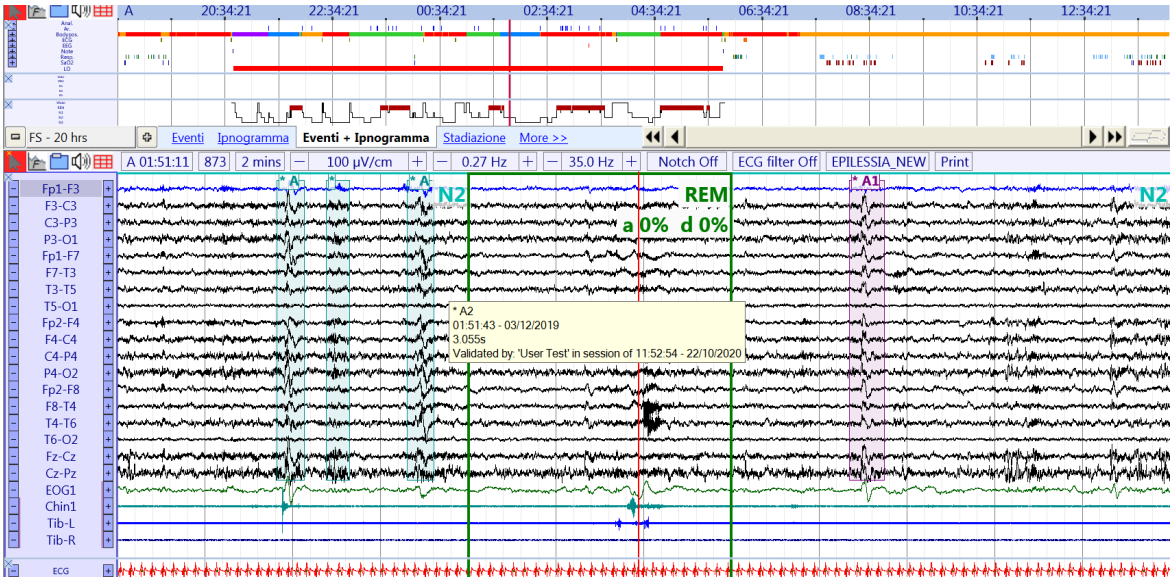

MIC_EEG_00000260.txt
SEQUENZA cicli contenuti 2 durata 43,120521 s
09/11/2019 04:40:53
09/11/2019 04:41:36
SEQUENZA cicli contenuti 4 durata 137,742671 s
09/11/2019 04:45:01
09/11/2019 04:47:19
SEQUENZA cicli contenuti 2 durata 91,397394 s
09/11/2019 04:48:44
09/11/2019 04:50:15
SEQUENZA cicli contenuti 8 durata 370,543974 s
09/11/2019 05:02:49
09/11/2019 05:08:59
SEQUENZA cicli contenuti 3 durata 70,495114 s
09/11/2019 05:12:39
09/11/2019 05:13:50
09/11/2019 05:15:59
09/11/2019 05:17:17
SEQUENZA cicli contenuti 5 durata 97,074918 s
09/11/2019 05:23:51
09/11/2019 05:25:28
SEQUENZA cicli contenuti 3 durata 127,52443 s
09/11/2019 06:50:18
SEQUENZA cicli contenuti 8 durata 177,635179 s
09/11/2019 07:01:18
09/11/2019 07:04:15
SEQUENZA cicli contenuti 2 durata 36,957654 s
09/11/2019 07:11:23
09/11/2019 07:12:00
TEMPO CAP A1 IN N1: 22,1319218333333
TEMPO CAP A1 IN N2: 8,845439733333333
TEMPO CAP A1 IN N3: 61,18089033333333
TEMPO CAP A2 IN N1: 2,64250813333333
TEMPO CAP A2 IN N2: 10,2274158833333
TEMPO CAP A2 IN N3: 13,9350162833333
TEMPO CAP A3 IN N1: 8,91758958333333
TEMPO CAP A3 IN N2: 17,33262755
TEMPO CAP A3 IN N3: 23,0458197666667
NUMERO SEQUENZE IN N1: 2
NUMERO SEQUENZE IN N2: 14
NUMERO SEQUENZE IN N3: 17
DURATA MEDIA SEQUENZE IN N1: 16,846009775
DURATA MEDIA SEQUENZE IN N2: 2,6003916547619


A 05:23:42
1354
2 mins
100 µV/cm
0.27 Hz
30.0 Hz
Notch On
ECG filter Off
EPILESSIA_NEW
Print
Fp1-F3
F3-C3
C3-P3
P3-O1
Fp1-F7
F7-T3
T3-T5
T5-O1
Fp2-F4
F4-C4
C4-P4
P4-O2
Fp2-F8
F8-T4
T4-T6
T6-O2
Fz-Cz
Cz-Pz
EOG1
Chin1
Tib-L
Tib-R
ECG
* A1
05:23:51 - 09/11/2019
1.974s
Validated by: 'User Test' in session of 21:40:49 - 18/03/2020
2 mins
Eventi
Ipnogramma
Eventi + Ipnogramma
Stadiazione *
More >>


A 05:25:12
1357
2 mins
100 µV/cm
0.27 Hz
30.0 Hz
Notch On
ECG filter Off
EPILESSIA_NEW
Print
-2.8 uV
0.07 Hz
1m15s
2 mins
Eventi
Ipnogramma
Eventi + Ipnogramma
Stadiazione *
More >>

Another important condition for the detection of the sequence is its interruption if a 30-seconds epoch of wake or REM occurs in the interval between two A-phases: even if the interval between the two phases is less than 60 seconds, the sequence ends with the B-phases that precedes the last A before the wake or REM epoch. Then another condition is added to the program: the algorithm makes a control and if the time that separates the two A-phases is also contained into a Wake or a REM sequence then the sequence is interrupted.

As showed in figure, this particular “challenging” situation for the algorithm is correctly detected and the sequence is interrupted by the NREM epoch.



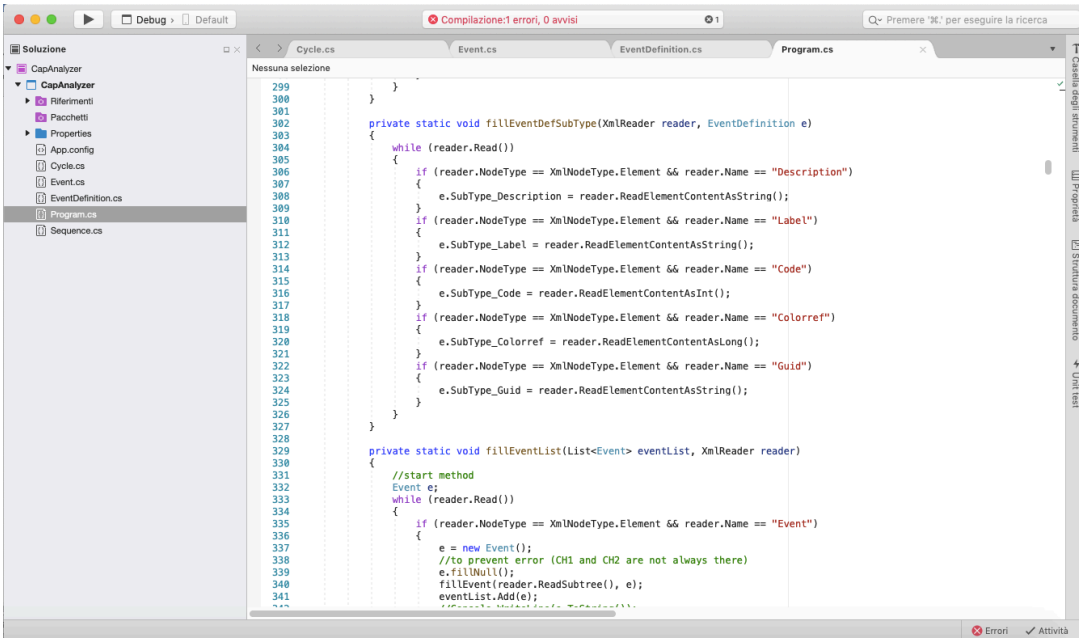

```
SEQUENZA cicli contenuti        8       durata  186,166123 s
02/12/2019 20:56:31
02/12/2019 20:59:37
SEQUENZA cicli contenuti        2       durata  14,833876 s
03/12/2019 01:51:29
03/12/2019 01:51:43
```

The sequence correctly detected starts at 01:51:29 and ends at 01:51:43. If there was any REM time between the third A2 phase and the A1 shown in figure, then the sequence would have continued, but it is interrupted at 01:51:43 with the beginning of the third A2 phase.

The particular situation occurs rarely but it is important because A-phases are more frequent during those unstable transition states and it could lead to errors in the calculation of CAP time.

How the software works

In its first phases of its creation the software worked as an independent external tool that could be run only with Microsoft Visual Studio.



This is an example of the first version of the software code compiled with Visual Studio.

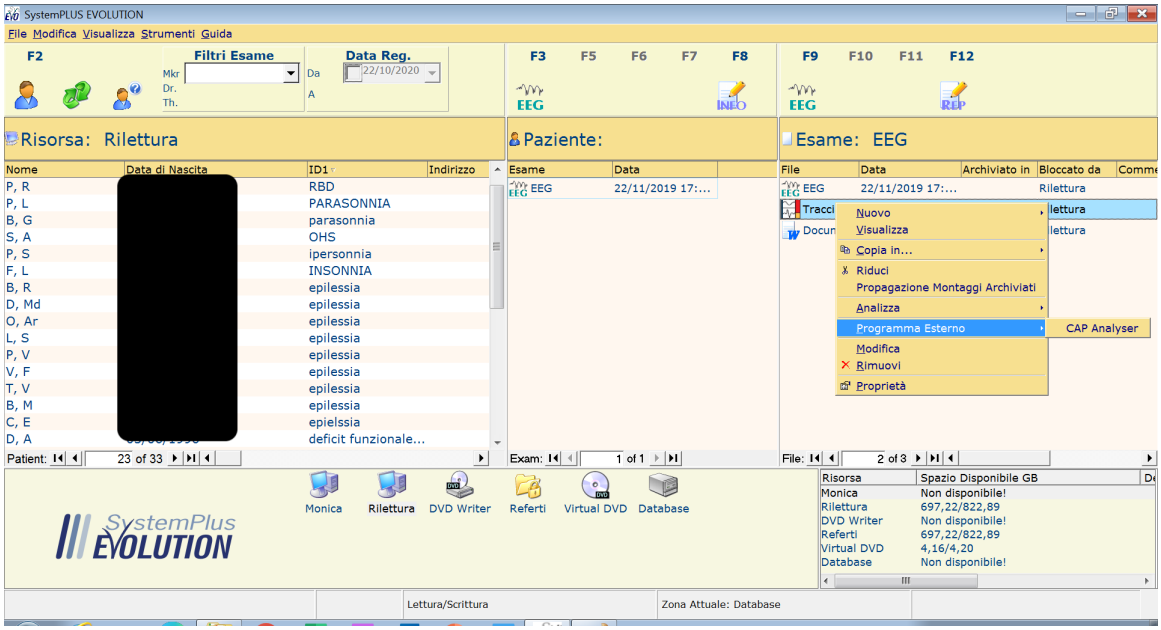
The last step of all the creation process was the integration of the software in Systemplus Evolution®.

The PSG is analysed with SleepRT™, all the scoring of sleep stages and the A-Phases events are done with this program.

Here is an example of how to run the software:

After all the analysis made with SleepRT™ for the sleep staging and the individuation of all A-phases the event file is automatically created as an .xml.

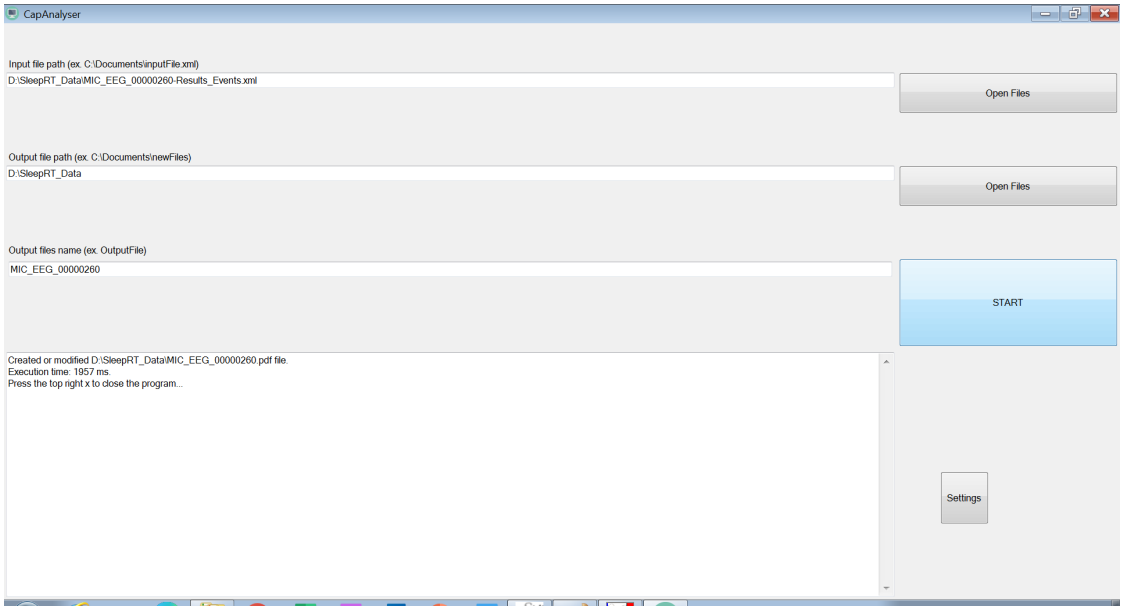
From the main page of Systemplus Evolution with a right click on the SleepRT™ analysis select “external program” and it shows all the plugins presents on that specific configuration.



Then the window of the “Capitalize” program pops-up: In the first line it is shown the input file that the software with analyse which is automatically retrieved.

Then the other are the selected output files with their name and archiving destination.

Clicking Start the program runs. As we can see the execution time is very rapid.



CAP report

At this point the report is created and saved in two formats, a raw ".txt" and a ".pdf" with all the parameters detected in a grid.

This is an example of the ".pdf" report created by CAP analyser.

Stadi del sonno	Tempo [minuti]	Tempo CAP [minuti]	CAP Rate [%]
N1	33,5	3,7	11
N2	172	68,5	39,8
N3	149,5	100,7	67,3
NREM	355	168,3	47,4

Stadi del sonno	Durata media fase A [secondi]	Durata media fase B [secondi]	Durata media fase A+B [secondi]	Cicli CAP	Indice CAP [cicli/ora]
N1	7,2	24,4	31,6	7	12,5
N2	7,7	23,9	31,6	130	45,3
N3	6,8	17,3	24,2	250	100,3
NREM	7,1	19,7	26,8	345	65,4

Stadi del sonno	Numero di sequenze	Durata media seq. [minuti]	Max. durata di seq. [minuti]	Cicli in sequenza [%]	Cicli in sequenza
N1	2	16,8	27,7		94
N2	14	2,6	10,3		66
N3	17	5,8	22,5		218
NREM	33	5,1	27,7	97,7	378

Parameters of the report.

All the parameters are automatically calculated by the program.

All parameters are calculated for total NREM time (last row) and for different NREM. A Cycle is assigned to the specific sleep stage if its A occurs during that sleep stage.

Here is a description of all the parameters listed in the image in the order of the columns that are shown in the picture.

- TIME (min) [tempo in minuti]: this is the duration of NREM, N1, N2, N3 expressed in minutes
- CAP TIME [Tempo CAP]: total duration of CAP time, CAP time is the total time occupied by CAP sequences.
- CAP RATE%: percentage of NREM occupied by CAP TIME. This is the most important parameter for the evaluation of CAP in clinical practice.
- MEAN A-PHASES DURATION [durata media fase A]: expressed in seconds
- MEAN B PHASES DURATION [durata media fase B]: expressed in seconds
- MEAN CYCLE DURATION [durata media fase A+B]: expressed in seconds
- NUMBER OF CYCLES [numero di cicli]: total number of CAP cycles.
- CAP INDEX [numero di cicli per ora]: is an index that indicates the number of cycles for every hour of NREM sleep.
- TOTAL NUMBER OF SEQUENCES
- MEAN DURATION OF SEQUENCES [durata media seq.]: expressed in minutes
- MAXIMUM DURATION OF A SEQUENCE[durata massima sequenza]: expressed in minutes
- CYCLES IN SEQUENCE [cicli in sequenza]: number and percentage of cycles that enters into a CAP sequence.

All the statistics are also divided for the different A-phases (A1,A2,A3).

Statistiche CAP - Fase A3

Stadi del sonno	Tempo [minuti]	Tempo CAP [minuti]	CAP Rate [%]
N1	33,5	8,9	26,6
N2	172	17,3	10,1
N3	149,5	23	15,4
NREM	355	49,3	13,9

Stadi del sonno	Durata media fase A [secondi]	Durata media fase B [secondi]	Durata media fase A+B [secondi]	Cicli CAP	Indice CAP [cicli/ora]
N1	7,2	24,4	31,6	7	12,5
N2	11,3	24,7	35,9	64	22,3
N3	14,9	19	33,9	17	6,8
NREM	11,5	28,1	34,3	74	12,5

Validation of the software CAP Analyzer

In order to validate the results of the analysis performed by the software "CAP analyser" the PhD student used a pool of six PSG exams that were previously analysed with a software provided with another PSG analysis software: Natus Remlogic®.

The PSG traces were converted in EDF (European DATA Format file) in order to open them with SleepRT™.

Using the event file ".txt" extracted from the analysis of those exams with REMlogic in which the starting time of the A-phase event and its duration in seconds are noted the PhD student manually marked all the A-Phases in SleepRT™.

The results of the analysis with Remlogic® and of the analysis made with SleepRT™ using CAP analysed were compared using Prism Graphpad 8® software.

Correlation coefficient r was calculated using nonparametric Spearman correlation.

PARAMETER	SPEARMAN CORRELATION COEFFICIENT (R)
CAP TIME	1,00
CAP RATE	1,00
MEAN B-PHASES DURATION	0,98
NUMBER OF SEQUENCES DETECTED	1,00
CAP INDEX	1,00

Table 1 Correlation of parameters analysed with "CAP analyser" with same parameters calculated by Remlogic®

The parameters listed showed an almost perfect coefficient of correlation between the two software. It must be taken into account that the fact that all the A-phases were manually rescored by the PhD student ,therefore the length and the starting of the A-phases and consequently of the B-phases detected by the software is not exactly the same but may differ in terms of milliseconds.

Moreover, a visual and manual validation was performed.

As described in the previous paragraphs, the PhD student, being a clinical expert with training in CAP scoring, supervised step by step the phases of the creation of the software verifying that all the cycles and sequences identified by "CAP analyser" corresponded with those identified visually in the PSG traces.

Conclusions and future perspective

CAP analyser is a new feature for the analysis of CAP with Micromed® software of EEG and PSG acquisition System Plus Evolution, Brain Quick and SleepRT™.

The validation of the parameters performed by a clinical expert opens for the use of the software for both clinical practice and research.

"CAP Analyzer" is currently being used in our Sleep Medicine Research Centre at University of Cagliari for the evaluation of CAP in patients with DOA and SHE. This analysis will help characterize sleep in the two different pathologies and will be useful for the differential diagnosis.

The introduction of this analysis in Systemplus/Brainquick® will allow a more extensive use of the CAP analysis in the different sleep labs, being Micromed®'s tools among the most used worldwide in sleep laboratories and neurophysiology clinics.

"CAP analyser" future is to be fully integrated in the next versions of Brain Quick and SleepRT™ and it will be possible to run it directly from the main screen of the PSG reading software.

CAP scoring is a very time consuming and error prone process.

The integration with a program for the automatic detection of A-phases is desirable: some algorithms have shown promising results.[31]

A future project is to develop and integrate automatic detection of A-phases in CAP analyser. Although a completely automatic algorithm that performs all the analysis without human intervention would be the most desirable result, even the introduction of a computer assisted analysis that requires only a visual verification and eventual manual correction from the operator would be extremely useful for a more rapid analysis.

PART 2: AUTOMATIC ANALYSIS OF MUSCLE ACTIVITY DURING REM AND NREM SLEEP

The second part of this thesis is focused on the use of automatic analysis of muscle EMG tone during both REM and NREM sleep.

Analysis of muscle tone during sleep: manual and automatic methods

The pattern of muscular activity during sleep was first characterized by Jouvet in 1964,[5] which described a progressive reduction of muscular EMG tone and activity starting from the maximum during wakefulness and its reduction trough different sleep stages, until the complete muscular atonia during REM sleep.
Motoneuronal activity is regulated by the neurophysiological mechanisms that control NREM and REM sleep. Whether there are several description and hypothesis for the phenomena regulating REM sleep muscular atonia, the neurophysiological basis of muscular regulation during NREM sleep are still unclear.

The recording of muscle activity in a PSG is performed with the use of an electromyography channel.
The muscle used for the recording in a standard PSG are the submental antigravity muscles and the anterior tibialis muscles for the detection of leg movements.
Chin EMG is recorded with two electrodes positioned in the submentalis area below the mandible referred to an electrode positioned in the midline of the chin.
The qualitative and quantitative analysis of chin muscle tone is a mandatory requirement for the evaluation of the preserved REM sleep atonia in patients with a suspect of RBD (REM sleep behaviour disorder): REM sleep without atonia (RSWA) is a key feature for this diagnosis. RSWA is characterized by the absence of physiological atonia during REM sleep with either a sustained or intermittent elevation of chin muscle tone activity or an excessive phasic activation.[54]

Several scoring methods have been developed for the quantification of RSWA both manual and automatic, the gold standard is still represented by the manual evaluation, but there are few methods that are validated and widely used for this purpose.

The first visual method to be accepted for clinical practice was developed by Lapierre and Montplaisir in 1992.[8] The authors scored the 20-seconds epochs as tonic if more than 50% of the epoch was occupied by an EMG activity that was twice the background activity or 10 μV. For the scoring of phasic activity the 30-s phasic is divided into 2-seconds mini-epochs and is scored as phasic if contains an EG activity lasting from 0,1s to 10s that exceeds four times the amplitude of background EMG.[55] If more than 30% of 20 seconds epochs are scored as tonic and more than 15% 2-seconds epochs are scored as phasic then RSWA is defined.[56] This method is validated for the diagnosis of idiopathic RBD and RBD associated to Parkinson's Disease.

The other visual method that defines a gold standard for the evaluation of RSWA was developed by Innsbruck and Barcelona group (SINBAR Group). With this method, other than EMG chin channel, it is also assessed with the combinate activity of the Flexor Digitorum Superficialis (FDS) muscles bilaterally, which augmented the accuracy of the analysis.[57,58] The definition of tonic epochs follows the definition of Lapierre and Montplaisir, phasic activity is assessed in 3 seconds mini epochs ad is defined as a burst of activity exceedingly twice the EMG background activity and lasting 0,5-5 seconds. Every 3-seconds mini epoch is also classified as "any chin activity" if contains either tonic, phasic or both within the same mini epoch.[7] Phasic activity is assessed using both chin and FDS muscles ,whether tonic activity chin only.[58]

The cut-off value for the diagnosis of RSWA related to both idiopathic and PD associated RBD is >32% epochs of containing 3-seconds epoch and 27% of 30 seconds of "any EMG chin" activity combined with phasic activity of FDS.[7] SINBAR criteria are the standard indicated by AASM in their scoring manual and classification of sleep disorders.[16,46]

Visual methods are very time consuming procedures and requires particular expertise, therefore is an error prone procedure and it is subject to an inter rater variability.[59]

Several computer assisted algorithms have been developed for the analysis of RSWA muscle activity during sleep but to date most of the studies are referred to the one developed and validated by R. Ferri, defined as “REM atonia index”(RAI).[57]

This method was developed in Italy and is based on the automatic analysis of EMG chin activity only [9,60], its use is validated for the diagnosis of RBD in Parkinson’s disease with an accuracy similar to the manual methods.[61,62]

In this algorithm the EMG signal is digitally band-pass filtered at 10–100Hz, with a notch filter at 50 Hz and rectified. Each 30-s sleep epoch included in the analysis is divided into 1-second mini-epochs, the average amplitude of the rectified chin EMG signal is calculated for each mini-epoch: the values of the chin EMG signal amplitude in mini-epoch are used to compute the percentage of values in the following 20 amplitude (amp) classes, expressed in μV: amp ≤ 1, 1 < amp ≤ 2 until >20.[9]

High values of the first class (amp ≤ 1) indicate muscle atonia, whereas phasic and tonic activations are expected to increase the value of the other classes.

Atonia index (AI) is computed for all sleep stages accordingly to the formula (AI = amp ≤ 1/(100–1 < amp ≤ 2).

Mathematically it can vary from 0 to 1 and it reflects the degree of preponderance of the first class of amplitude, the one that reflects complete atonia. Atonia index of 1 indicates complete atonia, whether AI of 0 indicates the complete absence of EMG atonia.

AI can vary from 0 (absence of mini-epochs with amp ≤ 1 that is complete absence of EMG atonia) to 1 (all mini-epochs with amp ≤ 1 or stable EMG atonia in the epoch.[9]

The program also constructs Graphics of the distribution histogram of mini epochs amplitude of the number of movements (divided similarly in duration classes of 1,2,3...>20 seconds) detected are created by the software. In both kind of histograms a more intense representation of the columns in the left part means a more a prominent phasic activity whether the columns on the right represents tonic activity.[63] RAI values below 0,8 are strongly suggestive of RSWA.[9]

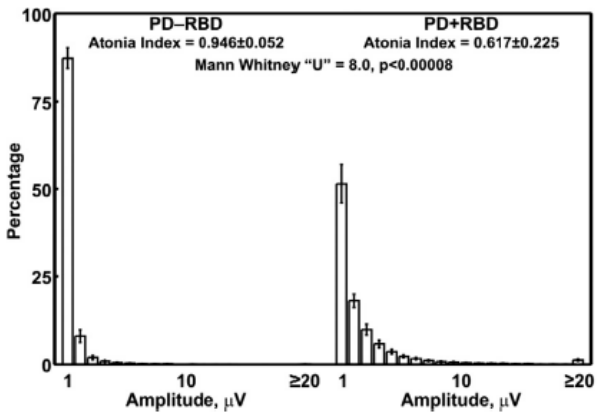


Figure 6 Histogram of distribution of the amplitude of the mini epochs. R.Ferri et al Sleep Medicine 2012

This analysis is not only performed during REM sleep but provides also an index of muscle activation for all the other sleep stages.

To date, analysis of muscle activity during NREM sleep was performed only in three studies and this remain still an unexplored territory .

Bliwise used its method, the Phasic electromyographic metric (PEM) for the evaluation of chin and limbs muscle activity in patients with PD. This method, which is a modified version of Lapierre and Montplaisir criteria, evaluates the presence of phasic activity only, and it was applied evaluating REM and NREM without differentiating the different phases. They found an increased PEM in both REM and NREM sleep in those patients, although no difference was found between PD patients with and without RBD.[64] The patients with akinetic/rigid syndrome had a higher PEM activity compared to tremor predominant patients.

Muscle activity during NREM sleep was also studied by Hanif and its group: both PD and iRBD patients were found to have a higher chin and anterior tibialis muscle activity during NREM compared to healthy controls, with the highest rate found in iRBD patients in all NREM sleep stages.[65] Moreover Schenck in its description of 96 cases of RBD recorder an incidence of both periodic and aperiodic limb movements in patients with RBD, hypothesizing that RBD disorder may not only be confined as a REM manifestation.[66]

The evaluation of muscle activity during NREM sleep is still an unexplored topic, and its evaluation may provide useful insights on the neurophysiological mechanisms of its regulation or highlight new marker of diagnostic value in different pathologies.

REM Sleep Behavior Disorder

The main application of muscle tone analysis is in the evaluation of loss of muscle atonia during REM sleep in patients with suspected Rem Sleep Behavior Disorder (RBD).

RBD is a sleep disorder characterized by loss of normal sleep atonia during REM sleep associated with dream enacting behavior.[67]

RBD may present as an isolated symptom without any particular cause (idiopathic RBD), but is more frequently associated to neurodegenerative disorders, in particular alpha synucleinopathies.[68] RBD may precede by many years the manifestation of the alpha synycleinopathies, a vast majority of patients presenting with idiopathic RBD will develop Parkinson, MSA and Lewy body dementia the years following this diagnosis (about 90% risk at 14 years of follow up).[69]

RBD may also be present in up to 60% of patients with Narcolepsy type 1[70] and recently its presence has been confirmed in patients with post-traumatic stress disorder.[71]

RBD and synucleinopathies association

Synucleynopathies are neurodegenerative disorders characterized by the formation of aggregated misfolded alpha synuclein that is deposited in different part of the central and peripheral nervous system.

Those group of neurodegenerative disorders is constituted by Parkinson's Disease Multiple System Atrophy (MSA) and Lewy body dementia.[72]

Parkinson's disease (PD) is a neurodegenerative disease involving the dopaminergic system in the brain. It is the second most common neurodegenerative disorder and involves 1% of the population above 60 years old.[73]

Neuropathological main finding in PD is an early loss of dopaminergic neuron of the substantia nigra pars compacta with dopaminergic denervation of the striatum, resulting in a dopaminergic deficit in the basal ganglia and extrapyramidal system.[74]

This neuronal death leads to an imbalance of dopaminergic system that act on motor controls but also has a wider influence on other circuits that influence cognition and impulse control. Parkinson is characterized by a wide variety of motor and non-motor symptoms. The hallmark motor symptoms of Parkinson's disease are the presence of resting tremor, bradykinesia and rigidity.[75] A wide variety of non-motor symptoms is present in PD in association to motor symptoms: sensory alterations, autonomic dysfunctions, vision impairment, sleep disorders, cognitive impairment and neuropsychiatric alterations. Those symptoms may occur during the disease course or even precede by many years the appearance of motor symptoms.[76]

Multiple System Atrophy is the most rapidly progressive of synucleinopathies and it is characterized by the deposit of alpha synuclein in oligodendroglial cells forming glial cytoplasmic inclusions (GCI).[77]

The mean incidence of MSA over the age of 50 is 3 cases per 100,000 person-years and its prevalence is of 1.9–4.4 cases per 100,000 person-year.[78]

It is characterized by the presence of dysautonomia, parkinsonism, cerebellar ataxia, and pyramidal signs in any combination.[79]

Differential diagnosis between PD and MSA, especially in the MSA-Parkinsonism (MSA-P) may be challenging and there is a lively continuous research of markers that can help in the differentiation.

RBD is observed in about 50 % in PD and in more than 80% of MSA patients.[80] It may be a prodromic symptom of both diseases, anticipating even of many years the appearance of the core symptoms.[81]

Presence of RBD in PD is a predictive factor for a poorer prognosis in Parkinson's disease.[82]

Sleep state dissociation

Sleep state dissociation consists in a simultaneous presence of features from different states of being.[10]

Loss of normal atonia during REM sleep and its behavioral correlate of RBD is an example of dissociated state arising from REM sleeps, with an admixture of features from different states of being, namely an intrusion of elements of wakefulness into REM sleep.[10]

Furthermore, the occurrence of motor behavioral episode occurring in NREM sleep associated with intrusion of REM phasic activity and rapid eye movements during slow wave sleep has been anecdotally described in a PD patient.[6] The presence of movements such as sudden trunk or limb jerks during NREM sleep in MSA patients, associated with an increased index of periodic Limb Movements during sleep (PLMS), was observed, as an expression of altered motor control during sleep in this condition.[83] Actually, dissociation of sleep states may be observed in advanced PD and MSA.[84]

Facial activity during sleep

Only few studies have assessed the presence of facial muscle activity during REM sleep, especially in relationship to dreaming and its emotional content. In premature children smiling has been described during the active sleep, which is the precursor of REM sleep, and the smiling activity continues randomly during sleep through the first months until it disappears after the first year of life. Only few studies have explored facial activity during sleep in adults.

A study conducted in healthy volunteers using both visual analysis of facial expressions using video-polysomnography (vPSG) and electromyographic recording (EMG) in mimic muscles (zygomatic, orbicularis and corrugator), showed the presence of activity of these muscles during all sleep stages, which was significantly higher during REM sleep, poor during N1 and N2 stages and almost absent during N3 delta sleep.[85] Duration of muscle activation was longer in REM sleep and in N2 stage than in the other stages.
Recently, Cle et al. analysed the occurrence of smiling expression during sleep in 174 subjects with REM and NREM parasomnias and healthy controls. Smiling during NREM sleep was very rare and occurred mostly in parasomnia patients, particularly in RBD, while only in one healthy control).[86]

FACIAL MUSCLES ACTIVITY AND MUSCLE TONE DURING SLEEP IN PATIENTS WHIT MULTIPLE SYSTEM ATROPHY AND PARKINSON'S DISEASE

Hypotheses and aims of the study

Our study is based on the fortuitous observation of a prominent facial activity during nocturnal vPSG in both NREM and REM sleep in some patients affected by MSA with and without RBD. We then hypothesized that the presence of facial activity during NREM might be found in MSA and in PD but not in healthy controls, as a manifestation of sleep state dissociation associated to the neurodegenerative process.

The aim of this study was to assess the presence of facial activity and to quantify the chin muscle activity during NREM and REM sleep in PD and MSA compared to healthy controls.

Materials and methods

Subjects

One hundred and one consecutive video-polysomnography (vPSG) recordings of PD patients recorded at the Sleep Medicine Centre at the University Hospital in Clermont-Ferrand, France and 12 v-PSG of MSA patients recorded at the same centre, plus 3 recorded at Sleep Medicine Centre at University of Cagliari (Italy) were selected for this study. Patients with PD were diagnosed according to MDS criteria[87] and MSA diagnosis were assessed accordingly to criteria proposed by American Academy of Neurology consensus.[88]

Control subjects were selected from a pool of consecutive recordings of individual who underwent to video-polysomnography for other suspected sleep problems and were from the same pool of age of PD and MSA patients; patients with an history of NREM parasomnia were excluded.

Patients and controls who did not show REM sleep (< 5 min) during their vPSG, or whose face was not clearly visible during the majority of the video-recording time were excluded.

As the main focus of this study was facial activity in NREM sleep and because MSA is a rare disease, for the analysis of the facial muscle activity in MSA, we decided to not exclude 1 MSA patient who did not achieve REM sleep.

Finally, the video recordings of 38 PD patients, 11 MSA patients and 13 controls were analysed.

All video-PSG were scored by a neurologist expert in sleep medicine.

Clinical and demographic data including disease duration, LED dose, history of RBD at the moment of the recording, were gathered from clinical letters.

Analysis of facial activity

All the recordings were anonymized.

Videos were extracted from vPSG and examined by the PhD student who was blinded to the clinical condition and to sleep staging. In order to detect movements, full night

video was preliminarily watched at augmented speed (10x), that was believed to be the maximum speed at which all small movements were detectable. Then, every episode was analysed at normal speed. Every facial movements detected was noted down and classified according to six main categories:

- **Eyes closing/opening**: including all squinting movements and definite opening/closing of eyelids
- **Frowning of eyebrows**
- **Raising of eyebrows**
- **"Smiling":** including every movement of lips corner raising similar to smiling, both bilateral and unilateral
- **Other mouth movements**: complex movement such as chewing or lips smacking, excluding movements linked to normal respiratory activity
- **"Strained face":** a particular expression involving both superior and inferior part of the face that results in a scrunched up and tensed expression.

After examining all videos, vPSG recordings were unblinded and the examinator went through the scoring in order to assess the stage of every movement noted.

Muscle Tone Analysis

Automatic quantification of chin EMG activity was performed according to a validated automatic scoring algorithm, by means of the HypnoLab software (SWS-Soft®, Italy).
The chin EMG signal is digitally band-pass filtered at 10–100Hz, with a notch filter at 50 Hz and rectified. Each 30-s sleep epoch included in the analysis is divided into 1-second mini-epochs, and the average amplitude of the rectified chin EMG signal is calculated for each mini-epoch: the values of the chin EMG signal amplitude in mini-epoch are used to compute the percentage of values in the following 20 amplitude (amp) classes, expressed in μV: amp ≤ 1, 1 < amp ≤ 2 until >20
High values of the first class (amp ≤ 1) indicate muscle atonia, whereas phasic and tonic activations are expected to increase the value of the other classes.

Atonia index is computed for all sleep stages accordingly to the formula (AI = amp ≤ 1/(100–1 < amp ≤ 2).

Mathematically it can vary from 0 to 1 and it reflects the degree of preponderance of the first class of amplitude, the one that reflects complete atonia. Atonia index of 1 indicates complete atonia, whether AI of 0 indicates the complete absence of EMG atonia.

AI can vary from 0 (absence of mini-epochs with amp ≤ 1 that is complete absence of EMG atonia) to 1 (all mini-epochs with amp ≤ 1 or stable EMG atonia in the epoch.[9]

Atonia index was computed for N1, N2, N3 and REM sleep stages.

This method is a validated method for the quantification of Submentalis chin EMG activity for the evaluation of Rem Sleep Without Atonia (RSWA) in the suspect of RBD.[61,62]

Statistical analysis

Statistical analysis was performed using Stata software (version 15, StataCorp, College Station, Texas, USA). Data were presented as mean and standard deviation and as median and interquartile range. The Shapiro-Wilk test was used to test the assumption of distribution normality for quantitative parameters. When the inflate of zeros was too high, it was decided to report results with mean and standard deviation. The comparisons between pathology groups were performed using ANOVA or Kruskal-Wallis test when the assumptions of ANOVA are not met. The homoscedasticity was analysed using Bartlett test. When appropriate (omnibus p-value less than 0.05), post-hoc test for two-by-two multiple comparisons were carried out taking into account correction of the type I error, i.e., Tukey-Kramer after ANOVA and Dunn after Kruskal-Wallis. Then, multivariable analyses were realized adjusting univariate results on age and REM or NREM sleep duration. More precisely, linear multiple regressions were performed, applying a Sidak's type I error correction for multiple comparisons between pathology groups. The results were expressed as Hedges' effect size (ES) and 95% confidence

intervals estimated according to multivariable results and were interpreted according to Cohen's rules of thumb which defined effect-size bounds as: small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8: grossly perceptible and therefore large). Furthermore, comparisons between groups for categorical data were done using Chi-squared or Fisher's exact tests, followed when appropriate by Marascuilo post-hoc test. The statistical tests were two-sided, with type I error at 0.05. As these analyses could be considered as exploratory, individual p-values have been reported without applying any mathematical correction but paiding on specific attention to the magnitude of differences (i.e. ES), according to several works reported in the literature like those discussed by Bender an Lange.[89]

Results

Population

Clinical and demographical data are shown in table 2. There was no difference between the three groups in terms of age, although MSA patients had a lower mean age than PD patients and controls but the difference was not significantly. No between-group difference was observed in sex distribution. Patients with MSA and PD have comparable mean LEDD. As expected, MSA disease duration was shorter compared to PD group.

Table 2 Clinical and Demographics

	MSA	PD	CTRL	p-value
Age (mean,sd)	60.81±8.34	66.9, ±9.05	67.5±6.4	0.107
Men n,%	4 (36.6%)	25 (65.8%)	7 (53.8%)	0.307
Women n,%	7 (63.3)	13 (34.2%)	6 (46.2)	
TOT LED (n sd)	554.22 ± 479.24	696.39± 460.07	0	
Disease duration years	1.9 ± 1.1	7.6 ± 5.5		

PSG features are shown in table 2. Total sleep time (TST), Sleep Efficiency (SE) and Wake after sleep onset (WASO) were similar between groups. No differences were found in percentages of NREM sleep stages, while REM sleep duration and percentage were reduced in PD compared to the other groups. No significant difference was observed in Apnea-Hypopnea index (AHI). Periodic limb movement (PLM) index was significantly different between the three groups with the highest rate in MSA patients (51,59), compared to 36,75 in PD groups and in 9,65 of control group. (p=0,0081). Twenty-five out of 38 (63%) PD patients and 10/11 (90%) of MSA patients had a diagnosis of RBD accordingly to ICSD-3 criteria.[46]

Table 3 PSG features

	MSA	**PD**	**CTRL**	**P-value**
TST	349.09 ±117.31	345.52±61.59	380.15± 60.72	0.2494
SE	68.96 ±23.74	75.05±13.10	80,92 ± 10,88	
Awakenings N°	19.55 ±12.57	29.47 ±19.62	18.41± 8.83	0.0702
Arousal index	10.50 ±7.58	7.82± 4.96	2.85± 2.62	0.0029*
WASO	99.81± 59.15	87.06±67.07	69.69± 40.49	0.4676
N1%	7.55 ± 2.64	9.17 ± 7.40	7.08 ± 5.52	0.5201
N2%	57.38 ± 10.92	59.14 ± 13.94	47.00 ± 5.63	0.05
N3%	22.51 ± 11.99	21.13 ± 10.49	26.25 ± 12.26	0.4
NREM sleep duration (min)	297,5±103,4	308,8±57	320,7±42,76	0,76
REM sleep %	13,05±8,5	10,52±6,3	19,44±12,55	0,02
REM sleep duration (min)	51,59±42,88	36,75±23,12	56,70±29,70	0,011
AHI	11.7 ± 12.6	6.0± 10.5	5.3± 5.0	0.1271
PLMS	51.14±48.35	23.34± 28.99	9.65± 20.01	*0.0081**
RBD n(%)	10 (90%)	25 (63%)	0 (0%)	

Facial movements

An hourly index of detected facial movements was calculated for all type of movements. Only the total movements index was considered for statistical purpose because the single indexes for each type of facial movements were too small. Total facial movements index during NREM sleep was 8.25 **±** 16.70 in MSA, 2.10 **±** 2.13 in PD, 0.022**±** 0.29 in HC, and was higher in PD patients compared to controls (p<0.001); During REM the total facial movement index was 18,28**±**17.54 for MSA group, 7.41**±**11.09 for PD and 0.269±0 .60 in control group, with a significant difference between with the two group compared to controls (PD vs CTRL p=0,01 and MSA vs CTRL 0,44).

Results of the analysis of each type of facial movements during NREM sleep are shown in table 3. In most of the HC subjects no facial movements were detected during NREM sleep, therefore mean number for every category was <1.
The mean total number of facial movements was significantly different between the three groups (MSA=26,27± 37,01, PD=11.053**±**12.64, HC=1.23**±**1.641; p=0.0001). Pairwise comparison showed a significant difference between both PD patients (p<0,0001) and control group, and MSA patients (p<0,0001) and HC, but not between PD and MSA patients (p=0,24).

For each different movement category during NREM sleep, pairwise comparison showed that MSA patients had an increased number of movements compared to healthy controls. PD and MSA patients did not show significant difference in eyes closing (p=0,556), eyebrows raising (p=0,559), eyebrows frowning (p=0,418), and movements of the mouth (p=0,20). However, the facial movements “strained face” (p=0,004) and “smiling” (p=0,035) were significantly more frequent in MSA patients compared to PD.
After adjusting for the presence of RBD, only the difference for “strained face” between MSA and PD remained significant (p=0,031), while “smiling” did not (p=0,38).

Table 4 Number of episodes of facial movements during NREM sleep

Results are expressed as mean± standard deviation. Results of the univariate analysis e and multivariate analysis. Results of the multivariate analysis are expressed in term of Effect Size , 95% Confidence interval (CI) and p value. Comparison between the three groups were adjusted for age and duration of NREM sleep. A and B represents comparison between PD and MSA vs Healthy Controls. C represents comparison between PD group vs MSA group and D represents the same comparison adjusted for the presence of RBD.

				Univariate Analysis	Multivariate Analysis A= PD vs. HC B= MSA vs. HC C= PD vs. MSA D= PD vs. MSA, adjusted for RBD
Type of facial movements	**MSA (n=11)**	**PD (n=38)**	**HC (n=13)**	**p-value**	**Effect size [CI 95%], p value**
Eyes closing	5.82±10.79	2.53±2.81	0.69±1.31	**0.0359***	**A : ES=0,36 [0.39 ; 0.69] p=0,029** B : **ES=0,66** [0.06 ; 1.27] p=**0,032*** C : ES=0,10 [-0.42 ; 0,23] p=0.556 D : ES=0 [0.33;0.33] p=0.996
Frowning eyebrows	0.82± 0.87	0.62 ±1.03	0	**0.0190***	**A : ES= 0.37[0;0.70] p=0.026** B : **ES= 0.73 [0.13;1.33] p=0.019*** C : ES=0.13[-0.46;1.19] p=0.418 D : ES=0.21[0.04;0.96]p=0.229
Raising eyebrows	5.27 ± 4.63	5.24± 8.47	0.30 ± 0.48	**0.0007***	A: **ES=0.61**[0.28;0.93] p=**0.000*** B: **ES=0.82** [0.21;1.42] **p=0.001*** C: ES= 0.10 [-0.42; 0.23] p=0.559 D: ES= 0.01[-0.33;0.32] p = 0.09
Smiling	2.89± 5.09	0.62± 1.63	0.08 ± 0.28	**0.0291***	A : ES=0.19.[-0.52;0.52] p=0.24 B : **ES=0.82** [0.21;1.42] p= **0.009*** C : **ES=-0.55** [-0.68; -0.03] **p= 0.035** D: ES= 0.19 [0.04;1.0]p= 0.38
Mouth movements	3.09± 5.26	1.13± 2.34	0.08± 0.27	0.0534*	A : ES=0.24 [-0.08;0.57]. 0.083 B : **ES =1.11** [0.50;1.71] p**=0.019*** C : ES=0.43 [-0.82;0.17] p=0.20 D : ES= 0.31[-0.59 ; -0.03]p= 0.496
Strained face	8.36± 20.30	0.87± 1.80	0.07± 0.28	**0.0051***	A: ES=0.24[-0.08;0.57] p=0.144 B : **ES=1.11** [0.50;1.71] p=**0.001*** C : **ES=0.49** [- 0.82; -0.17] p=**0.004*** D : ES = **0.36** [-0.59 ; -0.03]p= **0.031***
Total	26.27± 37.01	11.053 ±12.63	1.23 ± 1.64	**0.0001***	**A: ES=1.23 [0.70 ;1.75] p<0.0001*** B: **ES=1.37 [0.76 ;1.97] p<0.0001*** C: ES= 0.19 [-0.51;0.14] p=0.248 D: ES= 0.09 [;0.042 ;0.23] p=0.56

Table 4 Univariate and multivariate analyses of the number of episodes of facial movements during REM sleep

Results are expressed as mean± standard deviation. Results of the univariate analysis e and multivariate analysis. Results of the multivariate analysis are expressed in term of Effect Size , 95% Confidence interval (CI) and p value. Comparison between the three groups were adjusted for age and duration of REM sleep. A and B represents comparison between PD and MSA vs Healthy Controls. C represents comparison between PD group vs MSA group and D represents the same comparison adjusted for the presence of RBD.

				Univariate Analysis	**Multivariate Analysis** **A= PD vs HC** **B= MSA vs HC** **C= PD vs MSA** **D= PD vs MSA adjusted for RBD**
Type of activity	MSA (n=10) A	PD (n=38) B	HC (n=13) C	p-value	Effect size e [CI 95%], p
Eyes Closing	1.7 ± 2.41	1.18±2.06	0.15±0.38	0.09	**A : ES= 0.53[0.21;0.86]p=0.0019** **B :ES= 0.72[0.9;1.36]p=0.026** C :ES= 0.04 [-0.28;0.37] p=0.791 D ES =: 0.07[-0.26;0.39] p=0.687
Eyebrows frowning	0.8 ± 1.88	0.5 ± 1.47	0	0.14	A ES=0.29[-0.04-0.61]p=0.08 B ES=0.57 [0.06;1.21 p=0.075 C= ES-0.82[-0.41 ;0.24] p=0.616 D = ES= 0.01[-0.33;32] p=0.9
Eyebrows rising	9.8 ± 17.84	2.39 ± 4.86	0	0.005	A : ES=0.57[0.24;0.89]p=0.001 B : ES=1.33[0.69;1.96]p<0.0001 C : ES 0.28[-0.61;0.04] 0.09 D =ES 0.12[0.45 ;0.21] p=0.45
Smiling	2.5 ± 3.95	0.26± 0 .75	0	0.0003	**A: ES=0.35[0.28;0.68]p=0.034** **B: ES=1.57[0.94 ;2.21]==<0.0001** C: ES= 0.61[-0.94; -0.29] p=<0.0001 **D ES= 0.44 [-0.76; -0.11] p=0.01**
Mouth movements	1.4 ± 2.01	0.32±1.07	0.08± 0.28	0.0119	A: ES= 0.08[-0.24;0.41] p=0.602 **B:** ES= **0.85[0.22-1.49] p=0.009** **C :** ES= **0.43[0.75;0.10] p=0.011** D : ES= 0.31[-0.63; -0.02]p=0.066
Strained Face	2.79 ± 4.24	0.57 ± 1.67	0	0.0001	**A : ES =0.35[0.02-0.67]p=0.037** **B : ES= 1.36 [0.72;1.99] p<000.1** C ES=-0.49[-0.82;0.17] p=0.004 **D :** ES= **0.34[-0.67 ;-0.02] p=0.04**
Total	20.89 ± 24.62	5.24± 9.34	0.23± 0.44	<0.0001	**A : ES= 0.68[0.35;1.00]p=<0.0001** **B : ES= 1.61 [0.97;2.24] <0.0001** **C : ES=0.36[-0.50 ;-0.03]p=0.032** D ES= 0.22[-0.44 ;0.11]p=0.185

As in NREM sleep, also in REM sleep most of healthy controls did not manifested any facial movement, therefore the mean number for each movement category was below 1.

Pairwise comparison showed that MSA patients had an increased number of all types of movements compared to healthy controls, except for “eyebrows frowning”. PD and MSA patients did not show significant difference in eyes closing (p=0,556), eyebrows raising (p= 0,559), eyebrows frowning (p=0,418), and movements of the mouth (p=0,20). However, during REM sleep, facial movements as “strained face” and “smiling” were significantly more frequent in MSA compared to PD group as during NREM, and the difference was still significant after adjusting for the presence of RBD (p=0,04 and p=0,01 respectively).

Atonia index

Table 5 Univariate and multivariate analyses of Atonia index in MSA, PD and HC

				Univariate Analysis	**Multivariate Analysis** **A= PD VS. HC** **B= MSA VS. HC** **C= PD VS. MSA** **D= PD VS. MSA, adjusted for RBD**
Sleep stage	**MSA (n=12)**	**PD (n=38)**	**HC (n=10)**	**P-value**	**Effect size e [CI 95%], p**
N1	0.26 ± 0.22	0.43 ± 0.24	0.51 ± 0.19	0.030	A: ES= 0.19[-0.51 ; 0.14] p=0.25 **B: ES =1.07[-1.63 ;-0.1.36]** p= **0.003** C: ES= 0.43[0.13 ;0.78] p=0.007 ** **D: ES = 0.39[0.06 ; 0.72]** p=**0.02**
N2	0.33 ± 0.21	0.61 ± 0.21	0.73 ± 0.15	0.0007	A: ES=0.29[-0.62 ;0.12] 0.077 **B:** ES=**1.8 [-2.44 ;-1.17]<0.0001*** **C: ES=0.87[0.54;1.19]0.0000**** **D:**ES=**0.76 [0.44;1.09]** p=**0.02**

N3	0.58 ± 0.26	0.75 ± 0.21	0.89 ± 0.08	0.06	A:ES= 0.29[-0.61;0.04]p=0.08 **B:** ES=**1.13[-1.76. ;0.50]p=0.011** C: ES= 0.87[0.10;0.75]p=0.012 D :ES=0.32[-0.01;0.65] p=0.054
REM	0.44 ± 0.27	0.67 ± 0.28	0.84 ± 0.14	0.024	A= ES=0.31[-0.64 ;0.022] p=0.065 **B=** ES=**1.04[-1.85; -0.55]** p=<**0.0001** C= ES=0.45 [-0.48;0.78] p=0.0070* D ES= 0.28[-0.05;0.61] p= 0.09

Results of the univariate analysis are expressed in term of Effect Size , 95% Confidence interval (CI) and p value. Confrontation of groups were adjusted for age and duration of NREM sleep. Group Comparison : A PDvs Healthy Controls., BMSA vs HC C represents confrontation of PD group vs MSA group and D represents the same confrontation adjusted for the presence of RBD.

Atonia index was lower in MSA compared to HC during every sleep stage except during N3.

No significant differences between PD and healthy controls were found.

MSA muscle tone was significantly different compared to PD patients in stages N1, N2 and REM, this difference remained significant after adjusting for the presence of RBD in NREM sleep but did not survived the adjustment for RBD presence.

Figure 7 distribution of Atonia index in different sleep stages, column express mean, whiskers SD

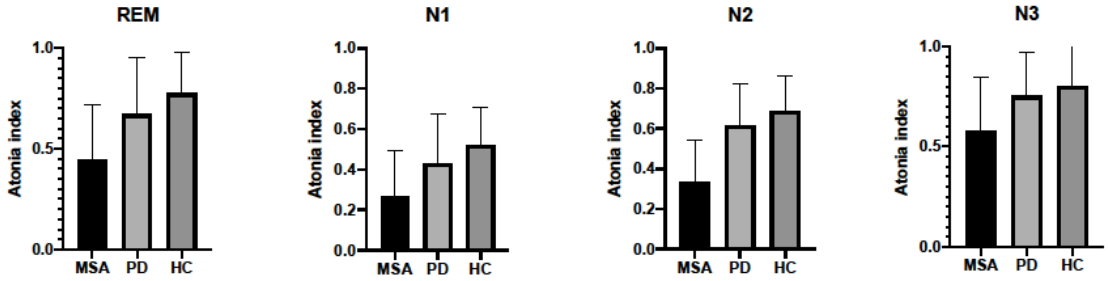


Figure 7 illustrate the distribution of Atonia index in different sleep stages in the three groups. A gradient of muscular activation was observed, which was higher, corresponding to lower AI, in MSA, and lower (i.e., higher AI) in HC in all sleep stages.

Discussion

Our findings open discussion for multiple interpretations.

The presence of the particular expression of “strained face” with the simultaneous contraction of both upper and lower parts of the face was a distinctive feature differentiating MSA from PD patients: indeed, during both NREM and REM sleep these movements were significantly more frequent in the MSA group compared to PD. During REM sleep, MSA patients also showed a higher frequency of “smiling”. This facial activity was increased in MSA compared to PD regardless to the presence of RBD.

Oro facial dystonia is a common presentation of dystonia in atypical parkinsonism,[90] considered a distinctive feature of MSA patients.[88] A common appearance is the combination of oromandibular dystonia, and its more common in combination with upper facial dystonia,[91] and sometimes this peculiar expression can resemble the contraction given by tetanus, and that lead to the definition of MSA risus sardonicus.[92] During sleep, dystonic movements tend to reduce in frequency and duration but do not completely disappear, however few data are available for dystonic movements in Parkinson and MSA during sleep.[93] The presence of facial movements during both NREM and REM sleep could be a nocturnal expression of this peculiar dystonia, although in our study the presence of diurnal oro-facial dystonia was not assessed.

Smiling during sleep has been poorly investigated in normal adults. One study found increased frequency of smiling during NREM sleep in patients with RBD compared to patients with NREM parasomnia and in non-parasomniac subjects.[86] During REM sleep, smiling was very frequently observed mostly in RBD patients.

In our study, during both NREM and REM sleep, facial movements as “eye closing”, eyebrows raising” and eyebrows frowning” were observed in both MSA and PD groups compared to healthy controls who almost never showed facial movements. Given the higher prevalence of RBD in these two groups of patients, these movements may be related to the presence of RBD.

From the analysis of muscle tone during the whole night of sleep, the physiological gradient of muscular activity that gradually decreases from wake throughout the

deepening of the stages (from NREM1 to NREM3) until REM atonia[94] was maintained in all three groups.

Although not significant, a trend for a more severe loss of atonia during REM sleep was observed in MSA compared to PD, with a lower RAI in this group of patients. The same was also observed in another cohort of MSA patients were a lower RAI was observed (together with a higher prevalence of sustained rather than phasic activation) as a possible marker of a more severe neurodegeneration.[60] Interestingly, in our study, this phenomenon was also found during NREM sleep, with the MSA patients presenting the lowest degree of muscle atonia throughout all the sleep stages compared to PD patients and healthy controls, with PD patients showing intermediate values of NREM AI between MSA and HC.

A few studies explored muscle activity during NREM in neurodegenerative disorders. Schenck et al. in its description of 96 cased of RBD found that that in RBD patients there was a prominence of aperiodic and periodic limb movements during both NREM and REM sleep compared to healthy controls, suggesting an altered motor control in both NREM and REM sleep in RBD.[66] One study using Phasic electromyographic metric (PEM) for the evaluation of chin and limbs muscle activity in patients with PD found an increased PEM in both REM and NREM sleep in those patients, although no difference was found between PD patients with and without RBD.[64] Muscle activity during NREM sleep was also studied by Hanif and its group: both PD and iRBD patients were found to have a higher chin and anterior tibialis muscle activity during NREM compared to healthy controls, with the highest rate found in iRBD patients in all NREM sleep stages.[65]

In our study, both facial movements and increased muscle tone were observed during NREM in MSA and PD patients compared to controls. After adjusting for the presence of RBD, the facial activity of “strained face” and the muscle tone during all NREM sleep stages were still significantly higher in MSA compared to PD. These results suggest that these abnormalities may be specifically related to this peculiar neurodegenerative disease rather than to the concomitant RBD. Future studies should confirm this finding. However, it must be taken into account that the vast majority of patients with MSA also have RBD which is a peculiar feature of the disease and the selection of a population of

MSA patient without RBD would be extremely difficult considering the prevalence in this disorder.[80]

On the other hand, in our study, the whole facial muscle activity during NREM, particularly those pertaining the upper face and including “eye closing”, “eyebrows raising” and “frowning” that were very much frequent in both MSA and PD who share the comorbidity with RBD, and almost absent in HC, may be modulated by the presence of RBD, as no difference were observed between MSA and PD.

Our findings can both be ascribed to the neurodegenerative process underlying Parkinson’s Disease and MSA, with the presence of RBD playing an influencing role in the dysregulation not only of REM sleep but also of NREM.

Mechanisms underlying the regulation of muscle activity during NREM sleep are not extensively studied like those of REM sleep. During NREM there the appearance of atonia before the onset of REM sleep starts five minutes before its beginning, and can be observed in 20 minutes of the subsequent NREM sleep episode,[95] and the presence of episodes of REM muscle atonia during NREM sleep is enhanced after selective REM sleep deprivation.[96] The hypothesis of a fluid control of NREM and REM rather than a rigid separate mechanisms was hypothesized by Nelsen with the concept of covert-REM.[97]

Therefore, if REM sleep atonia is impaired in PD and in other alpha-synucleinopathies associated to RBD, leading to an increased phasic and tonic muscle activity during REM sleep, it is conceivable that these muscle abnormalities may also emerge during NREM sleep.

On the other hand, the presence of both facial activity and muscle activation during NREM sleep may also be a manifestation of sleep state dissociation which is described in neurodegenerative disorders, including MSA and PD. Sleep state dissociation consist in a simultaneous presence of features of different states of being. For example, RBD is characterized by an intrusion of elements of wakefulness such as motor behaviors, into REM sleep.[84,10] Indeed, an evolution from RBD to frank status dissociatus, with motor activity observed during all sleep stages has been described in MSA.[98] Therefore, in our

MSA and PD patients, the presence of a higher number of facial movements detected in the video analysis may represent an early manifestation of state dissociation.
A longitudinal analysis of facial movements and muscle tone during NREM will provide information on the evolution of the sleep dissociation in those neurodegenerative conditions.

Conclusion

To the best of our knowledge this is the first study to assess and quantify the presence of facial movements during both REM and NREM sleep and the muscle tone during all sleep stages in patients with Multiple System Atrophy and Parkinson Disease.
Our results show the presence of an augmented facial activity in MSA patients compared to healthy controls in both REM and NREM sleep and the presence of a peculiar expression could be a red flag for the differential diagnosis between PD and MSA.
The alteration of muscle tone in PD and MSA compared to healthy controls is an important finding that open discussion for pathogenetic mechanisms of this phenomenon that can be related to the synucleinopathy or being an expression of the influence of RBD on NREM sleep.
Automated analysis of muscle activity by means of Atonia index calculation proved to be a useful and reliable method for the evaluation of muscle tone not only for REM sleep but also for NREM sleep.
Further studies on a population of PD patients with and without RBD, using this analysis will help to understand whether the sustained muscle activity is related to PD and MSA or to the associated RBD.
These studies may also provide new insights on the mechanisms of neurophysiological regulation of muscle tone during sleep.

CONCLUSIONS OF THE THESIS

The research work carried out in this present thesis underlines the importance of the cooperation between clinical experts with engineers and software programmers.
The software CAP analyser is the fruit of this work of collaboration: all the phases of the creation of this software were supervised by the PhD student, a clinical expert, that also cured all the validation of the software.
The development of a software for the analysis of CAP for Micromed® tools will help the diffusion of this analysis in several sleep labs worldwide, which is currently performed by only few centers and mostly for research purpose.
Future perspective on this work is the integration of the software with an automatic algorithm for the detection of A-phases on the EEG traces.
This will help to overcome two important problems with this procedure: the fact that requires highly trained operator, with an important inter rater and inter lab-variability and the fact that is an extremely time consuming procedure.

The automatic analysis of muscle tone during sleep through the calculation of atonia index is a validated method for the evaluation of RSWA for the diagnosis of RBD.
Manual methods such as SINBAR and Montreal Methods are still the gold standard proposed by the American Academy of Sleep medicine, but their use is very time consuming and has an important inter-rater variability.
The evaluation of muscle tone during NREM sleep would not be possible using manual methods, because it would take too many time and would be subject to the same rater variability of the other manual methods.
With this thesis we evaluated the feasibility of this analysis using the “Ferri’s atonia Index” applied to all sleep stages.
As noted from the results in the study described in this thesis, atonia index during NREM sleep stages reflects clearly the process of progressive reduction of muscle tone trough N1, N2, N3 sleep stages.

This may be an indicator of its reliability for the use of this muscle analysis in the whole night of sleep.

Moreover the study presented with this thesis showed a difference in chin muscle tone during NREM sleep between MSA and PD patients compared to Healthy Controls.

The finding of this study opens for a new point of view: there may be and alteration of muscle tone control in those patients that may be related to the pathology itself or to the presence of RBD, which may be a condition not only confined to REM sleep.

Future perspective is to use this method for the evaluation of muscle activity during NREM sleep in a larger population of Parkinson's patients, comparing the results between patients with and without comorbid RBD.

This will also help the understanding of the neurophysiological mechanisms of muscle control during NREM sleep, which is not widely explored such as those of REM sleep.

The particular industrial characterization of this PhD project that viewed me as PhD student with a background of medicine studies and Neurology specialization working with an industry was important occasion of professional enrichment.

This experience was of particular value because it allowed me to widen the field of my research which is usually confined to pure clinical research.

The experience in Micromed® was highly formative: not only did I work on the creation of the software, but I was also introduced to all the work of the Research and Development department. This allowed me to know in detail how the instruments that I use daily in my clinical practice are created, tested and validated for their use in clinical practice.

Knowing all the processes behind the creation of medical device software and hardware, and the requirements for validation for clinical use is extremely useful added value for a researcher.

Automatic and semiautomatic algorithms for the analysis of biological parameters will have in the future an always increasingly prominent role in research and clinical practice.

These features, even though not leading to a complete automatization of the whole analysis process of EEG or PSG would be of extreme help for the clinician and for the researcher, allowing an important spare of time, increasing the precision of the analysis and making feasible analysis of parameters that would be otherwise impossible, as we saw with the muscle tone analysis in NREM sleep in this thesis.

Bibliography

1. Terzano, M. G. *et al.* Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep. *Sleep Med.* **2**, 537–53 (2001).
2. Parrino, L., Ferri, R., Bruni, O. & Terzano, M. G. Cyclic alternating pattern (CAP): The marker of sleep instability. *Sleep Medicine Reviews* **16**, 27–45 (2012).
3. Terzano, M. G. & Parrino, L. Origin and significance of the cyclic alternating pattern (CAP). *Sleep Medicine Reviews* **4**, 101–123 (2000).
4. Parrino, L., Grassi, A. & Milioli, G. Cyclic alternating pattern in polysomnography: What is it and what does it mean? *Current Opinion in Pulmonary Medicine* **20**, 533–541 (2014).
5. Jouvet, M. The states of sleep. *Sci. Am.* **216**, (1967).
6. Dauvilliers, Y., Jennum, P. & Plazzi, G. Rapid eye movement sleep behavior disorder and rapid eye movement sleep without atonia in narcolepsy. *Sleep Med.* **14**, 775–781 (2013).
7. Frauscher, B. *et al.* Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. *Sleep* **35**, 835–847 (2012).
8. Lapierre, O. & Montplaisir, J. Polysomnographic features of REM sleep behavior disorder: Development of a scoring method. *Neurology* **42**, 1371–1374 (1992).
9. Ferri, R. *et al.* Improved computation of the atonia index in normal controls and patients with REM sleep behavior disorder. *Sleep Med.* **11**, 947–949 (2010).
10. Mahowald, M. W. & Schenck, C. H. Status dissociatus - A perspective on states of being. *Sleep* **14**, 69–79 (1991).
11. St Louis, E. K. & Boeve, B. F. REM Sleep Behavior Disorder: Diagnosis, Clinical Implications, and Future Directions. *Mayo Clinic Proceedings* **92**, 1723–1736 (2017).
12. American Academy of Sleep Medicine. *The AASM Manual for the Scoring of Sleep and Associated Events (Online/Print) Bundle*.
13. Halász, P. Chapter 55 The microstructure of sleep. *Suppl. Clin. Neurophysiol.* **57**, 521–533 (2004).

14. Terzano, M. G. *et al.* The cyclic alternating pattern as a physiologic component of normal NREM sleep. *Sleep* **8**, 137–145 (1985).
15. Smerieri, A., Parrino, L., Agosti, M., Ferri, R. & Terzano, M. G. Cyclic alternating pattern sequences and non-cyclic alternating pattern periods in human sleep. *Clin. Neurophysiol.* **118**, 2305–2313 (2007).
16. Berry, R. B. *et al.* The AASM Manual for the Scoring of Sleep and Associated Events.
17. Parrino, L., Ferri, R., Zucconi, M. & Fanfulla, F. Commentary from the Italian Association of Sleep Medicine on the AASM manual for the scoring of sleep and associated events: For debate and discussion. *Sleep Med.* **10**, 799–808 (2009).
18. Bricolo, A., Faccioli, F. & Turazzi, S. L'E.E.G. dans le coma traumatique aigu valeur diagnostique et pronostique. *Rev. Electroencephalogr. Neurophysiol. Clin.* **9**, 116–130 (1979).
19. Terzano, M. G., Parrino, L., Fioriti, G., Orofiamma, B. & Depoortere, H. Modifications of sleep structure induced by increasing levels of acoustic perturbation in normal subjects. *Electroencephalogr. Clin. Neurophysiol.* **76**, 29–38 (1990).
20. Halász, P. Hierarchy of micro-arousals and the microstructure of sleep. *Neurophysiologie Clinique* **28**, 461–475 (1998).
21. Terzano, M. G., Parrino, L. & Spaggiari, M. C. The cyclic alternating pattern sequences in the dynamic organization of sleep. *Electroencephalogr. Clin. Neurophysiol.* **69**, 437–447 (1988).
22. BORBÉLY, A. A. & ACHERMANN, P. Concepts and models of sleep regulation: an overview. *J. Sleep Res.* **1**, 63–79 (1992).
23. Terzano, M. G., Parrino, L., Boselli, M., Smerieri, A. & Spaggiari, M. C. CAP components and EEG synchronization in the first 3 sleep cycles. *Clin. Neurophysiol.* **111**, 283–290 (2000).
24. Terzano, M. G. *et al.* CAP and arousals are involved in the homeostatic and ultradian sleep processes. *J. Sleep Res.* **14**, 359–368 (2005).
25. Parrino, L., Boselli, M., Spaggiari, M. C., Smerieri, A. & Terzano, M. G. Cyclic

alternating pattern (CAP) in normal sleep: Polysomnographic parameters in different age groups. *Electroencephalogr. Clin. Neurophysiol.* **107**, 439–450 (1998).

26. Bruni, O. *et al.* Sleep cyclic alternating pattern in normal preschool-aged children. *Sleep* **28**, 220–230 (2005).
27. Bruni, O. *et al.* Sleep cyclic alternating pattern in normal school-age children. *Clin. Neurophysiol.* **113**, 1806–1814 (2002).
28. Terzano, M., Parrino, L., Fioriti, G., Spaggiari, M. C. & Piroli, A. Morphologic and functional features of cyclic alternating pattern (CAP) sequences in normal NREM sleep. *undefined* (1986).
29. Rosa, A., Alves, G. R., Brito, M., Lopes, M. C. & Tufik, S. Visual and automatic cyclic alternating pattern (CAP) scoring: inter-rater reliability study. *Arq. Neuropsiquiatr.* **64**, 578–81 (2006).
30. Ferri, R. *et al.* Inter-rater reliability of sleep cyclic alternating pattern (CAP) scoring and validation of a new computer-assisted CAP scoring method. *Clin. Neurophysiol.* **116**, 696–707 (2005).
31. Largo, R. *et al.* Visual and automatic classification of the cyclic alternating pattern in electroencephalography during sleep. *Brazilian J. Med. Biol. Res.* **52**, (2019).
32. Danker-Hopfe, H. *et al.* Interrater reliability for sleep scoring according to the Rechtschaffen & Kales and the new AASM standard. *J. Sleep Res.* **18**, 74–84 (2009).
33. Hartmann, S. & Baumert, M. Improved A-phase Detection of Cyclic Alternating Pattern Using Deep Learning. in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* **2019**, 1842–1845 (Institute of Electrical and Electronics Engineers Inc., 2019).
34. Ferri, R. *et al.* Inter-rater reliability of sleep cyclic alternating pattern (CAP) scoring and validation of a new computer-assisted CAP scoring method. **116**, 696–707 (2005).
35. Foldvary-Schaefer, N. & Grigg-Damberger, M. Sleep and epilepsy: What we

know, don't know, and need to know. in *Journal of Clinical Neurophysiology* **23**, 4–20 (2006).

36. Halász, P., Bódizs, R., Ujma, P. P., Fabó, D. & Szűcs, A. Strong relationship between NREM sleep, epilepsy and plastic functions — A conceptual review on the neurophysiology background. *Epilepsy Research* **150**, 95–105 (2019).
37. Lanigar, S. & Bandyopadhyay, S. Sleep and Epilepsy: A Complex Interplay. *Mo. Med.* **114**, 453–457 (2017).
38. Gigli, G. L. *et al.* Sleep Microstructure and EEG Epileptiform Activity in Patients with Juvenile Myoclonic Epilepsy. *Epilepsia* **33**, 799–804 (1992).
39. Parrino, L., Smerieri, A., Spaggiari, M. C. & Terzano, M. G. Cyclic alternating pattern (CAP) and epilepsy during sleep: How a physiological rhythm modulates a pathological event. *Clin. Neurophysiol.* **111**, (2000).
40. Tinuper, P. & Bisulli, F. From nocturnal frontal lobe epilepsy to Sleep-Related Hypermotor Epilepsy: A 35-year diagnostic challenge. *Seizure* **44**, 87–92 (2017).
41. Tinuper, P. *et al.* Definition and diagnostic criteria of sleep-related hypermotor epilepsy. *Neurology* **86**, 1834–1842 (2016).
42. Zucconi, M., Oldani, A., Smirne, S. & Ferini-Strambi, L. The macrostructure and microstructure of sleep in patients with autosomal dominant nocturnal frontal lobe epilepsy. *J. Clin. Neurophysiol.* **17**, 77–86 (2000).
43. Parrino, L. *et al.* Distinctive polysomnographic traits in nocturnal frontal lobe epilepsy. *Epilepsia* **53**, 1178–1184 (2012).
44. Terzaghi, M. *et al.* Coupling of minor motor events and epileptiform discharges with arousal fluctuations in NFLE. *Epilepsia* **49**, 670–676 (2008).
45. Gibbs, S. A. *et al.* Sleep-related epileptic behaviors and non-REM-related parasomnias: Insights from stereo-EEG. (2016). doi:10.1016/j.smrv.2015.05.002
46. American Academy of Sleep Medicine. *International classification of sleep disorders-third edition*. (American Academy of Sleep Medicine, 2014).
47. Castelnovo, A., Lopez, R., Proserpio, P., Nobili, L. & Dauvilliers, Y. NREM sleep parasomnias as disorders of sleep-state dissociation. *Nat. Rev. Neurol.* **14**, 1–12 (2018).

48. Baldini, T. *et al.* Clinical features and pathophysiology of disorders of arousal in adults: A window into the sleeping brain. *Front. Neurol.* **10**, 526 (2019).
49. Zucconi M, Oldani A, Ferini-Strambi L, S. S. Arousal fluctuations in non-rapid eye movement parasomnias: the role of cyclic alternating pattern as a measure of sleep instability. *J Clin Neurophysiol.* **12**, 147–154 (1995).
50. Guilleminault, C. *et al.* Non-REM-sleep instability in recurrent sleepwalking in pre-pubertal children. *Sleep Med.* **6**, 515–521 (2005).
51. Bruni, O. *et al.* NREM sleep instability in children with sleep terrors: The role of slow wave activity interruptions. *Clin. Neurophysiol.* **119**, 985–992 (2008).
52. Gaudreau, H., Joncas, S., Zadra, A. & Montplaisir, J. Dynamics of slow-wave activity during the NREM sleep of sleepwalkers and control subjects. *Sleep* **23**, 755–760 (2000).
53. Guilleminault, C., Kirisoglu, C., da Rosa, A. C., Lopes, C. & Chan, A. Sleepwalking, a disorder of NREM sleep instability. *Sleep Med.* **7**, 163–170 (2006).
54. Manual, C. The International Classification of Sleep Disorders. *Int. Classif.* **24**, 297 (2005).
55. Montplaisir, J. *et al.* Polysomnographic diagnosis of idiopathic REM sleep behavior disorder. *Mov. Disord.* **25**, 2044–2051 (2010).
56. McCarter, S. J. *et al.* Diagnostic REM sleep muscle activity thresholds in patients with idiopathic REM sleep behavior disorder with and without obstructive sleep apnea. *Sleep Med.* **33**, 23–29 (2017).
57. Frauscher, B., Ehrmann, L. & Högl, B. Defining muscle activities for assessment of rapid eye movement sleep behavior disorder: From a qualitative to a quantitative diagnostic level. *Sleep Med.* **14**, 729–733 (2013).
58. Frauscher, B. *et al.* Quantification of electromyographic activity during REM sleep in multiple muscles in REM sleep behavior disorder. *Sleep* **31**, 724–731 (2008).
59. Högl, B., Santamaria, J., Iranzo, A. & Stefani, A. Precision Medicine in Rapid Eye Movement Sleep Behavior Disorder. *Sleep Medicine Clinics* **14**, (2019).
60. FERRI, R. *et al.* A quantitative statistical analysis of the submentalis muscle EMG

amplitude during sleep in normal controls and patients with REM sleep behavior disorder. *J. Sleep Res.* **17**, 89–100 (2008).

61. Ferri, R., Gagnon, J. F., Postuma, R. B., Rundo, F. & Montplaisir, J. Y. Comparison between an automatic and a visual scoring method of the chin muscle tone during rapid eye movement sleep. *Sleep Med.* **15**, 661–665 (2014).
62. Figorilli, M. *et al.* Comparison between automatic and visual scorings of REM sleep without atonia for the diagnosis of REM sleep behavior disorder in Parkinson disease. *Sleep* **40**, (2017).
63. Ferri, R., Bruni, O., Fulda, S., Zucconi, M. & Plazzi, G. A quantitative analysis of the submentalis muscle electromyographic amplitude during rapid eye movement sleep across the lifespan. *J. Sleep Res.* **21**, 257–263 (2012).
64. Hanif, U., Trap, L., Jennum, P., Zoetmulder, M. & Sorensen, H. B. D. Quantification of muscle activity during sleep for patients with neurodegenerative diseases. in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* **2015**-**Novem**, 6002–6005 (2015).
65. Bliwise, D. L., Trotti, L. M., Greer, S. A., Juncos, J. J. & Rye, D. B. Phasic muscle activity in sleep and clinical features of parkinson disease. *Ann. Neurol.* **68**, 353–359 (2010).
66. Schenck, C. H., Hurwitz, T. D. & Mahowald, M. W. REM sleep behaviour disorder: An update on a series of 96 patients and a review of the world literature. in *Journal of Sleep Research* **2**, 224–231 (1993).
67. Schenck, C. H. & Mahowald, M. W. REM sleep behavior disorder: Clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP. *Sleep* **25**, 120–138 (2002).
68. Barone, D. A. & Henchcliffe, C. Rapid eye movement sleep behavior disorder and the link to alpha-synucleinopathies. *Clinical Neurophysiology* **129**, 1551–1564 (2018).
69. Iranzo, A. *et al.* Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: Study in 174 patients. *PLoS One* **9**, 89741 (2014).

70. Antelmi, E., Pizza, F., Franceschini, C., Ferri, R. & Plazzi, G. REM sleep behavior disorder in narcolepsy: A secondary form or an intrinsic feature? *Sleep Medicine Reviews* **50**, (2020).
71. Lee, E. *et al.* REM Sleep Behavior Disorder among Veterans with and without Post-Traumatic Stress Disorder. *Psychiatry Investig.* **17**, (2020).
72. Goedert, M., Jakes, R. & Spillantini, M. G. The Synucleinopathies: Twenty Years on. *Journal of Parkinson's Disease* **7**, S53–S71 (2017).
73. Tysnes, O. B. & Storstein, A. Epidemiology of Parkinson's disease. *Journal of Neural Transmission* **124**, 901–905 (2017).
74. Dickson, D. W. Neuropathology of Parkinson disease. *Park. Relat. Disord.* **46**, S30–S33 (2018).
75. Reich, S. G. & Savitt, J. M. Parkinson's Disease. *Medical Clinics of North America* **103**, 337–350 (2019).
76. Pfeiffer, R. F. Non-motor symptoms in Parkinson's disease. *Park. Relat. Disord.* **22**, S119–S122 (2016).
77. Palma, J. A., Norcliffe-Kaufmann, L. & Kaufmann, H. Diagnosis of multiple system atrophy. *Autonomic Neuroscience: Basic and Clinical* **211**, 15–25 (2018).
78. Erkkinen, M. G., Kim, M. O. & Geschwind, M. D. Clinical neurology and epidemiology of the major neurodegenerative diseases. *Cold Spring Harb. Perspect. Biol.* **10**, (2018).
79. Köllensperger, M. *et al.* Presentation, diagnosis, and management of multiple system atrophy in Europe: Final analysis of the European multiple system atrophy registry. *Mov. Disord.* **25**, 2604–2612 (2010).
80. Palma, J. A. *et al.* Prevalence of REM sleep behavior disorder in multiple system atrophy: A multicenter study and meta-analysis. *Clin. Auton. Res.* **25**, 69–75 (2015).
81. Iranzo, A., Santamaria, J. & Tolosa, E. The clinical and pathophysiological relevance of REM sleep behavior disorder in neurodegenerative diseases. *Sleep Medicine Reviews* **13**, 385–401 (2009).
82. Kim, Y. *et al.* REM sleep behavior disorder portends poor prognosis in

Parkinson's disease: A systematic review. *Journal of Clinical Neuroscience* **47**, 6–13 (2018).

83. Iranzo, A. *et al.* Characteristics of idiopathic REM sleep behavior disorder and that associated with MSA and PD. *Neurology* **65**, 247–252 (2005).
84. Antelmi, E. *et al.* From state dissociation to status dissociatus. *Sleep Medicine Reviews* **28**, 5–17 (2016).
85. RIVERA-GARCÍA, A. P., RAMÍREZ-SALADO, I., CORSI-CABRERA, M. & CALVO, J. M. Facial muscle activation during sleep and its relation to the rapid eye movements of REM sleep. *J. Sleep Res.* **20**, 82–91 (2011).
86. Clé, M. *et al.* Smiling asleep: A study of happy emotional expressions during adult sleep. *J. Sleep Res.* **28**, e12814 (2019).
87. Postuma, R. B. *et al.* MDS clinical diagnostic criteria for Parkinson's disease. *Movement Disorders* **30**, 1591–1601 (2015).
88. Gilman, S. *et al.* Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* **71**, 670–676 (2008).
89. Bender, R. & Lange, S. Adjusting for multiple testing - When and how? *J. Clin. Epidemiol.* **54**, 343–349 (2001).
90. Yoon, W. T. Comparison of dystonia between Parkinson's disease and atypical parkinsonism: The clinical usefulness of dystonia distribution and characteristics in the differential diagnosis of parkinsonism. *Neurol. Neurochir. Pol.* **52**, 48–53 (2018).
91. Thongchuam, Y., Panyakaew, P. & Bhidayasiri, R. Orofacial dystonia and asssociated bulbar symptoms in multiple system atrophy: A blinded video analysis. *J. Neurol. Sci.* **417**, (2020).
92. Wenning, G. K., Geser, F. & Poewe, W. The 'risus sardonicus' of multiple system atrophy [4]. *Movement Disorders* **18**, 1211 (2003).
93. Hertenstein, E. *et al.* Sleep in patients with primary dystonia: A systematic review on the state of research and perspectives. *Sleep Medicine Reviews* **26**, 95–107 (2015).
94. Brunner, D. P., Dijk, D. J. & Borbély, A. A. A quantitative analysis of phasic and

tonic submental EMG activity in human sleep. *Physiol. Behav.* **48**, 741–748 (1990).

95. Bliwise, D. *et al.* Facial Muscle Tonus During REM and NREM Sleep. *Psychophysiology* **11**, 497–508 (1974).

96. Werth, E., Achermann, P., Ly, A. A. B., Werth, P. & Achermann, A. A. Selective REM sleep deprivation during daytime. II. Muscle atonia in non-REM sleep. *Am J Physiol Regul. Integr. Comp Physiol* **283**, 527–532 (2002).

97. Nielsen, T. A. A review of mentation in REM and NREM sleep: 'Covert' REM sleep as a possible reconciliation of two opposing models. *Behav. Brain Sci.* **23**, 851–866 (2000).

98. Vetrugno, R. *et al.* Status dissociatus evolving from REM sleep behaviour disorder in multiple system atrophy. *Sleep Med.* **10**, 247–252 (2009).

La borsa di dottorato è stata cofinanziata con risorse del
Programma Operativo Nazionale Ricerca e Innovazione 2014-2020 (CCI 2014IT16M2OP005),
Fondo Sociale Europeo, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale"

UNIONE EUROPEA
Fondo Sociale Europeo