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**ANALYSIS OF CEREBRAL AND AUTONOMIC RESPONSE TO RESPIRATORY
EVENTS IN PATIENTS WITH SLEEP APNEA SYNDROME**

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| | |
|---|-----------|
| Abbreviations | 1 |
| Introduction..... | 3 |
| Object of the study | 8 |
| Materials..... | 8 |
| Inclusion Criteria:..... | 8 |
| Study Protocol | 8 |
| Section I: AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF CYCLIC ALTERNATING PATTERN | |
| Background..... | 11 |
| Materials..... | 11 |
| Methods | 12 |
| Scoring of Respiratory Events..... | 12 |
| Scoring of EEG..... | 13 |
| Scoring of PWA..... | 13 |
| Temporal relation between arousal responses (cerebral and autonomic) and respiratory events | 14 |
| Statistical analysis..... | 16 |
| Results | 16 |
| Sleep Parameters..... | 20 |
| Respiratory Events..... | 20 |
| Discussion | 25 |
| Sleep parameters..... | 25 |
| Cerebral and autonomic responses to the respiratory events..... | 25 |
| Limitations | 27 |
| Conclusions..... | 27 |
| Section II: AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF PULSE WAVE AMPLITUDE | |
| Background..... | 30 |
| Materials..... | 32 |
| Methods | 32 |
| Evaluation of the response (cerebral and autonomic) to respiratory events..... | 32 |
| Results | 33 |
| Additional scoring of the FL events with the AASM EEG arousal | 42 |
| Discussion | 46 |
| Limitations | 47 |
| Conclusions..... | 48 |
| Conclusions..... | 50 |

| | |
|---|-----------|
| Appendix: Introduction | 52 |
| CAP scoring rules | 52 |
| Recording techniques and montages | 54 |
| CAP A phases subtypes..... | 54 |
| PWA analysis..... | 56 |
| Appendix: Section I | 59 |
| Appendix: Section II | 67 |
| Bibliography | 71 |

ABBREVIATIONS

Abbreviations

PSG: Polysomnography,
OSAS: Obstructive Sleep Apnea Syndrome,
PM: Portable Monitoring,
EEG: Electroencephalographic traces,
AHI: Apnea Hypopnea Index,
RERA: Respiratory Events Related Arousal,
AASM: American Academy of Sleep Medicine,
CAP: Cyclic Alternating Pattern,
AP: Apnea,
H:Hypopnea,
FL: Flow Limitation,
OA: Obstructive Apnea,
CA: Central Apnea,
MA: Mixed Apnea,
NREM: Non Rapid Eye Movements,
REM: Rapid Eye Movement,.
TST: Total Sleep Time
WASO: Wake After Sleep Onset,
SE: Sleep Efficiency
PWA: Pulse Wave Amplitude,
PTT: Pulse Transit Time

INTRODUCTION

Introduction

Polysomnography (PSG) is the gold standard for quantifying respiratory events in patients with Obstructive Sleep Apnea Syndrome (OSAS). However it is considered an expensive and technically intense procedure. For these reasons portable monitoring (PM) is becoming an increasingly accepted cost-effective alternative approach¹⁻⁶. Although PM is less time consuming and can be applied to investigate not only OSAS but also other sleep disorders^{7,8}, it lacks neurological signals. The absence of electroencephalographic (EEG) traces precludes the identification of cortical arousals and the use of this criterion in the definition of respiratory events⁹.

The Apnea Hypopnea Index (AHI) is the most common parameter used for OSAS severity classification. However, some studies show that arousal scoring in OSAS patients is important to clarify the impact of this disease on sleep¹⁰⁻¹². In particular, Chandra et al.¹³ have demonstrated that patients with a high RERA (respiratory event related arousal) index, even in the setting of a low or normal AHI, may be exposed to elevated sympathetic tone during sleep.

The definition of arousal is controversial, and the currently applied American Academy of Sleep Medicine (AASM) criteria¹⁴ may underestimate more subtle alterations of sleep^{10,12,15}.

EEG arousal can be defined as an abrupt shift in frequencies including alpha, theta and/or frequencies greater than 16 Hz (but not spindles) that lasts at least 3 seconds, with at least 10 seconds of stable sleep preceding the change¹⁶ (Fig 0-1).

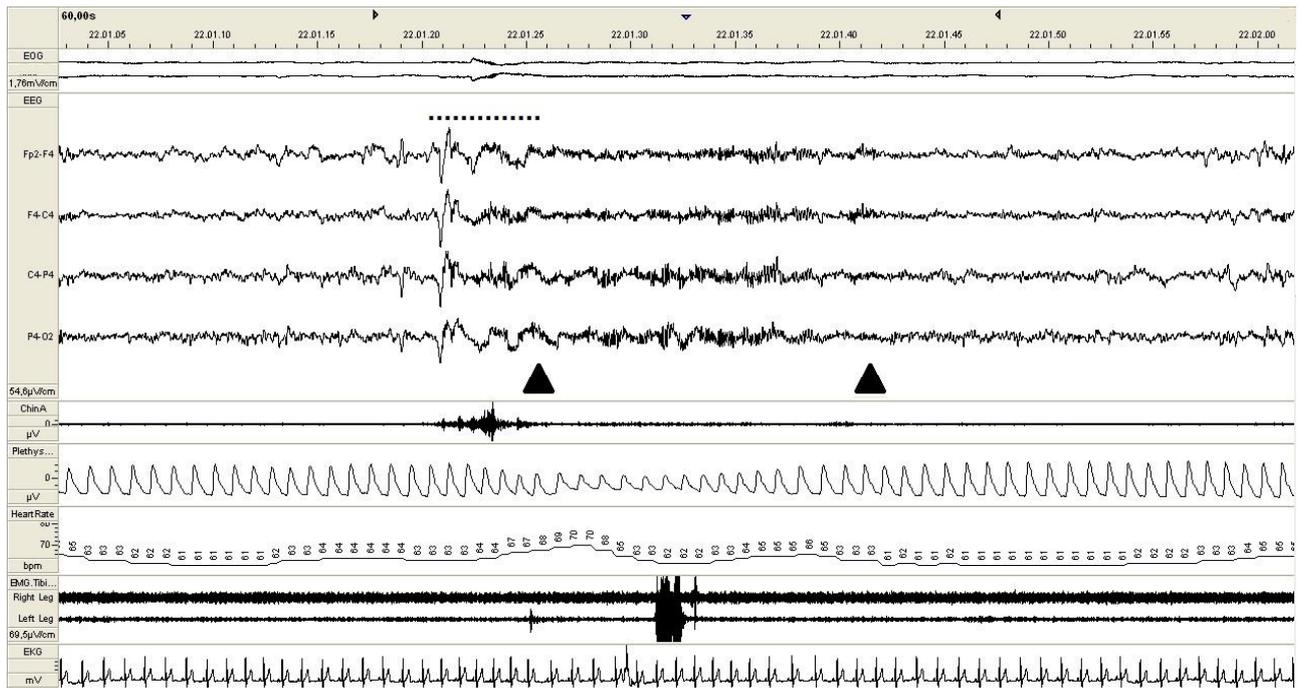


Figure 0- 1: EEG arousal in NREM sleep

EEG arousal according to AASM scoring criteria (indicated by the black triangles) The spot line shows the occurrence of slow wave activity preceding the conventional arousal, that heralds the onset of EEG desynchrony.

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

Beside the specific definition, the term arousal is also used as an equivalent of cerebral activation. Rechtschaffen & Kales (R&K) rules, established in 1968, excluded EEG arousals from conventional staging procedures¹⁷. In 1992, the American Sleep Disorders Association (ASDA; later named AASM, American Academy of Sleep Medicine) defined arousals as markers of sleep disruption representing a detrimental and harmful feature for sleep¹⁸. In the following years, however, a number of studies clarified that spontaneous arousals are natural guests of sleep and undergo a linear increase along the lifespan following the profile of maturation and aging¹⁹. Arousal scoring is now considered a fundamental process in staging classification as well as spindles and K-complexes. Nevertheless, current AASM rules still restrain sleep dynamics within rigid epochs, neglecting evidence that sleep is a continuous function that cannot be restricted to a static sequence of artificial segments. Actually, each 30-seconds epoch encompasses several short-time events carrying important information that disappear in the classical sleep staging reports.

The most comprehensive method for their detection and analysis is the so-called cyclic alternating pattern or CAP²⁰. Because CAP spans across long periods of non rapide eye movements (NREM) sleep, it overcomes the boundaries of standard rigid epochs and offers a dynamic contribution to the static framework of conventional scoring. CAP reveals and describes

the presence of a complex sleep microstructure, hidden but also perfectly integrated beneath the surface of conventional sleep macrostructure.

In OSAS patients, the multiple airflow interruptions followed by arousals show a 20- to 40-s cyclic pattern²¹. This periodicity of the OSAS-related phenomena recalls the physiological component of CAP²² (Fig 0-2).

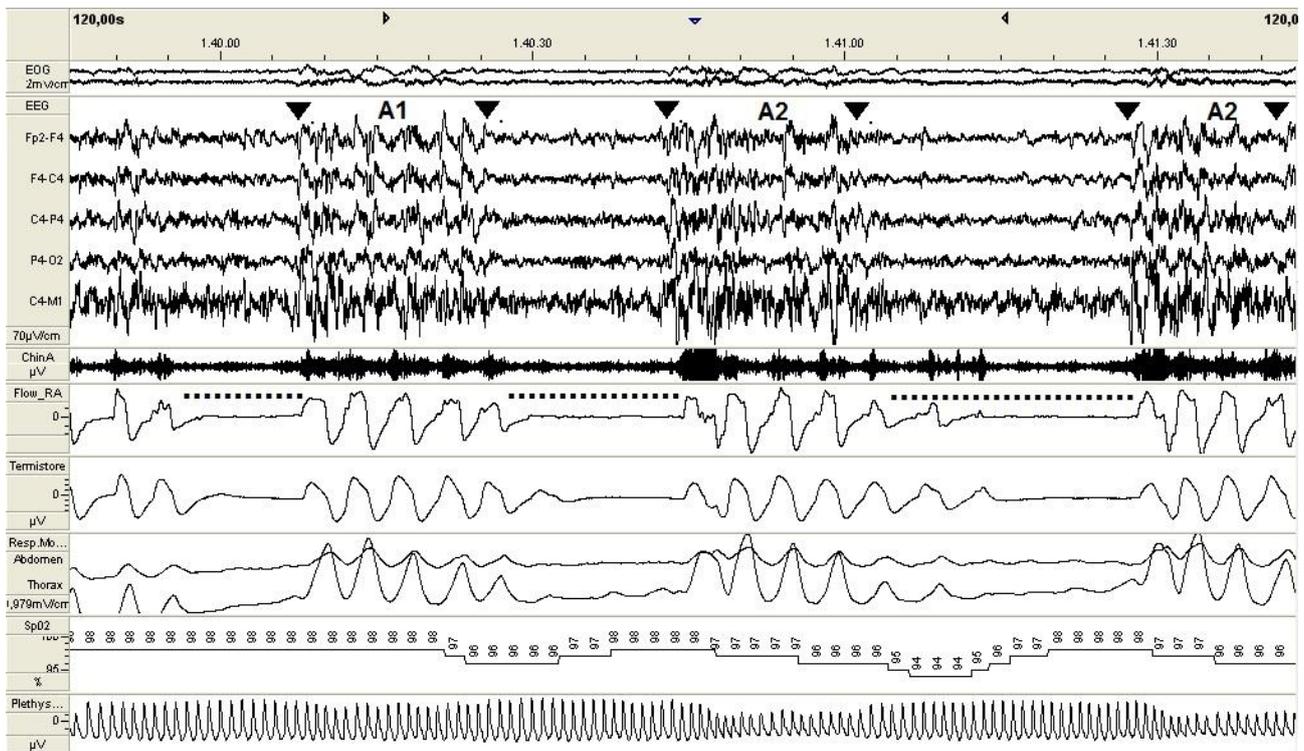


Figure 0- 2: Modulation of EEG response to respiratory events

Three examples of respiratory events (dotted lines) in which airway re-opening occur with CAP a phases (delimited by black triangles).

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

Three subtypes of A phases can be identified corresponding to different levels of activation²³: subtype A1 consists of high-voltage low-frequency EEG patterns associated with mild motor and neurovegetative activation; subtype A2, which is composed of rapid high-frequency EEG patterns preceded by or associated with slow high-amplitude waves, is linked with a moderate increase of muscle tone and cardiorespiratory rate; subtype A3, in which rapid high-frequency EEG patterns occupy at least 2/3 of the entire phase A length. Subtype A3 is generally coupled with a remarkable enhancement of muscle tone and cardiorespiratory rate²⁴.

In the CAP framework, the cerebral response is a complex phenomenon in which the arousal definition is more extensive compared to the AASM criteria¹⁴, incorporating not only the low-voltage high-frequency EEG bands such as alpha and beta (AASM arousal or subtypes A2 and A3

of CAP A phases), but also the high-voltage lower frequency activities such as theta and delta bands (A1 CAP A phases).

It is known that, compared to normal subjects, OSAS patients show enhanced amounts of CAP with the great majority of apnea (AP) and hypopnea (H) ending with CAP A phases, especially subtypes A2 and A3 subtypes²⁵

As a translation of fluctuating vigilance, CAP offers a favorable background for phasic and/or repetitive sleep-related manifestations. However, typical manifestations of secondary cortical events are also the respiratory effort-related arousals (RERA). More specifically, RERA are defined by obstructive upper airway airflow reductions (which do not meet the criteria of apnea or hypopnea) associated with progressive negative esophageal pressure lasting ≥ 10 s and culminating in an arousal²⁶ (Fig 0-3).

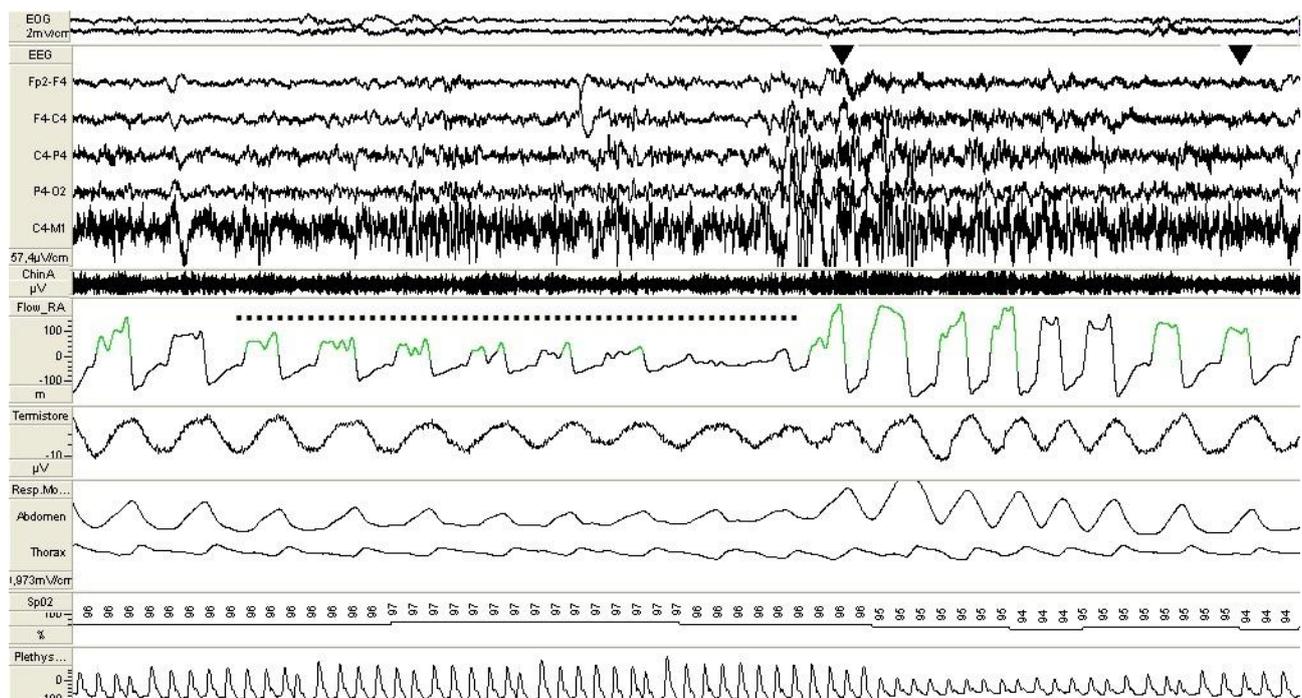


Figure 0- 3: Respiratory effort related arousal (RERA).

A sequence of breaths that do not meet criteria for an apnea or hypopnea by increasing respiratory effort or flattening of the nasal pressure waveform (indicated by the dotted line) leading to an EEG arousal from sleep (delimited by the black triangles).

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

RERA are increased both in OSAS and in UARS as a reaction of the sleeping brain to a repetitive breathing disturbance, contributing to excessive daytime sleepiness even in the absence of other

respiratory events^{27,28}. In general, the association between respiratory events and EEG arousals is more frequently reported in OSAS than in UARS. This is likely because OSAS subjects present increase in effort accompanied by apneas and hypopneas accompanied by oxygen saturation drops, thus requiring a more intense stimulus to arouse. Correlation between the number of arousals and daytime sleepiness in OSAS patients has been already reported^{29,30}, but the activating role of phasic delta activities during sleep should also be emphasized. There are literature reports that airway opening may occur with a predominant increase in delta power both in UARS³¹⁻³³ and OSAS³⁴. Involvement of either slow or fast EEG responses is based on the regulation of upper airway pathways, depending on sensory recruitment and response adequacy. The sleeping brain can solve respiratory challenges even without involving a cortical arousal. The latter is triggered only when thalamo-cortical structures fail to modulate breathing or when ascending reticular volleys are required to restore respiration³⁵.

The autonomic nervous system is also enhanced by EEG activations, which explains the greater increase in heart rate in the presence of EEG arousals. In any case, the problem is quantitative and not qualitative, in the sense that delta bursts can also determine heart rate acceleration and autonomic activation regardless of a concomitant EEG arousal^{36,37}. Generally, the slow and the fast components of EEG activation have different latencies, with the delta portion preceding the rapid activities³⁸. This probably determines a graduated impact on the autonomic system. The slow waves determine a softer vegetative reaction, which in certain pathologic conditions may be strong enough to overcome a disturbing factor, e.g. an obstructive event. Otherwise, the slow rhythms are immediately replaced by faster EEG activities, which guarantee a more powerful activation of autonomic functions. It is plausible that the effects on daytime function may not derive by a single A phase subtype, but more likely by the reciprocal amount and distribution of the whole CAP phenomenon. In OSAS patients effectively treated with nasal CPAP, therapy induces a significant reduction of CAP instability as well as daytime sleepiness. The improvement is associated with a robust curtailment of A3 subtypes and an expansion of A1 percentage³⁹.

In OSAS patients the presence of respiratory events not only induce arousals but also changes in autonomic markers such as heart rate (HR)^{36,40}, blood pressure⁴¹, peripheral arterial tonometry (PAT)⁴²⁻⁴⁴, pulse transit time (PTT)⁴⁵⁻⁴⁷, and photoplethysmography^{48,49}. Zacharia et Al.⁵⁰ confirmed that respiratory events, such as AP and H induce more changes in pulse wave amplitude (PWA) than changes in HR as measured by photoplethysmography. They also found more changes in PWA than HR after respiratory events without cortical arousal. Photoplethysmography non-invasively measures the relative absorption of red light and infrared light. Arterial blood flow pulsation passing through an artery modulates the light absorption and provokes a pulse wave easily convertible in an electrical signal readable during PSG. This parameter can be easily derived from conventional pulse oxymeters and does not bring any

alteration of sleep, as there is no variation of pressure at the fingertip. It has also been used as a marker of finger vasoconstriction⁵¹ and shown to be more sensitive than HR as an autonomic marker⁴⁸. The supplementary use of PWA improves detection of more subtle sleep events such as H but not AP, which are easily detected at first sight and increase in inter-observer agreement for detecting respiratory events but not EEG arousals⁵⁰.

In contrast, there are no available data on the relation between flow limitation (FL) events and PWA parameters.

Object of the study

The Aims of the present study were to evaluate the impact of respiratory events (including FL) on cortical and autonomic arousal response and to quantify the additional value of CAP and PWA for a more accurate detection of subtle respiratory events and sleep alterations in OSAS patients.

Materials

Medical records of OSAS patients, performed at the sleep laboratory of the Pulmonary Operative Unit, Dept of Thoracic Diseases, G.B. Morgagni–L.Pierantoni Hospital, Forlì, Italy between January 2012 and June 2012, were retrospectively revised.

Inclusion Criteria:

- Patients without central nervous system disease or other sleep disorders (i.e. insomnia, narcolepsy, restless leg syndrome...);
- Absence of psychoactive, cardiac, antihypertensive, or other drugs interfering with the sleep and autonomic system;
- Presence of five or more scoreable respiratory events for hour of sleep in the PM or PSG
- All patients had to provide written informed consent to participate in the study

Study Protocol

Nineteen OSAS subjects met the inclusion criteria. All patients underwent the nocturnal PSG in the sleep laboratory of the Pulmonary Operative Unit, Dept of Thoracic Diseases, G.B. Morgagni–L.Pierantoni Hospital, Forlì, Italy.

The PSG interpretation, carried out by two experienced sleep investigators (1 pulmonologist, 1 neurologist) was performed in four different steps:

1. scoring of respiratory events (AP, H and FL) based on visual analysis of respiratory traces;
2. scoring of EEG activity, applying both the AASM criteria and the CAP rules;
3. evaluation of autonomic activity by means of PWA phasic drops;
4. temporal relation between arousal responses (cerebral and vegetative) and respiratory events.

Only the events that met the agreement of both scorers were included in the data analysis.

OSAS patients were divided in two severity groups. According to a consensus opinion of the American Academy of Sleep Medicine Task Force ⁵², the mild group was composed of patients with an $5/h < AHI < 15/h$, while an $AHI > 15/h$ characterized the moderate-severe group.

Patients results were compared with those of normal age- balanced subjects, selected from the database of the Parma University Sleep Disorders Center.

Section I

AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF CYCLIC ALTERNATING PATTERN

Section I: AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF CYCLIC ALTERNATING PATTERN

Background

The arousal scoring in OSAS is important to clarify the impact of the disease on sleep but the currently applied AASM definition¹⁴ may underestimate the alterations of sleep. The scoring AASM arousal is also mandatory for the definition of subtle respiratory events such as RERA. However there are other EEG activation phenomena, such as A phases of CAP which are associated with respiratory events in NREM sleep.

CAP provides a more extensive interpretation of arousal compared to the AASM criteria. In the CAP framework, the cerebral response is a complex phenomenon in which the arousal definition is more extensive compared to the AASM criteria, as it incorporates not only the low-voltage high-frequency EEG bands such as alpha and beta (AASM arousal or subtypes A2 and A3 of CAP A phases), but also the high voltage lower frequency activities such as theta and delta waves (A1 CAP A phases)²³

It is also known that, compared to normal subjects, OSAS patients show enhanced amounts of CAP with the great majority of AP and H ending with CAP A phases, especially subtypes A2 and A3 subtypes²⁵. In contrast, there are no available data on the relation between FL events and CAP parameters and the impact of FL events on sleep is still unclear.

The present study aims at assessing in OSAS patients the impact of respiratory events (including FL events) on sleep and whether CAP represents an additional value, compared to the currently applied AASM arousal criteria, for a more accurate detection of subtle respiratory events and alterations of sleep in OSAS patients.

Materials

Nineteen PSG recordings of OSAS patients (males, mean age 54 ± 10) were analyzed. The full-night attended PSG was performed in each patient with Embla® Titanium (Embla Systems). No patient had prior diagnosis of a central nervous system disease or was taking psychoactive, cardiac, antihypertensive, or other drugs interfering with the autonomic system.

Sleep was recorded from F4-M1, C4-M1, O2-M1 derivations integrated by bipolar montages (Fp2-F4, F4-C4, C4-P4, P4-O2) used to optimize the scoring of CAP²⁴. PSG measures included also electrooculographic channels, submental electromyogram, activity of the right and left anterior tibialis muscles, oronasal thermal sensor, nasal air pressure transducer, thoracic and abdominal effort by respiratory inductive plethysmograph, electrocardiogram, body position, oxygen saturation and pulse wave amplitude (PWA), the last two recorded with finger photoplethysmogram.

Studies were performed at the sleep laboratory of the Pulmonary Operative Unit, Dept of Thoracic Diseases, G.B. Morgagni–L.Pierantoni Hospital, Forlì, Italy. The institutional review board approved the revision of PSG data.

Methods

The PSG interpretation, carried out by two experienced sleep investigators (1 pulmonologist, 1 neurologist) was performed in four different steps:

1. scoring of respiratory events (AP, H and FL) based on visual analysis of respiratory traces;
2. interpretation of EEG activity, in which arousal was defined applying both the AASM criteria¹⁴ and the CAP rules²⁴;
3. evaluation of autonomic activity by means of PWA phasic drops;
4. temporal relation between arousal responses (cerebral and autonomic) and respiratory events.

Only the events that met the agreement of both scorers were included in the data analysis.

OSAS patients were divided in two severity groups. According to a consensus opinion of the American Academy of Sleep Medicine Task Force⁵², the mild group was composed of patients with an $5/h < AHI < 15/h$, while an $AHI > 15/h$ characterized the moderate-severe group.

Scoring of Respiratory Events

The respiratory events were defined according to the AASM rules¹⁴.

Obstructive apnea (OA) was defined as a reduction in the oral thermistor signal to less than 10% of baseline, lasting at least 10 seconds with continued or increased respiratory effort.

Central apnea (CA) was identified when there was absence of respiratory effort.

Mixed apnea (MA) was scored when a respiratory effort was absent in the initial portion of the event, followed by resumption of inspiratory effort in the second portion of the event.

Hypopnea (H) was defined as a drop by $\geq 30\%$ in the nasal cannula flow lasting at least 10 seconds and associated with a $\geq 4\%$ desaturation.

FL event (FL) was defined as a sequence of breaths, not meeting criteria for AP or H, lasting at least 10 seconds characterized by increasing respiratory effort (assessed by inductance plethysmography) and/or by flattening of the inspiratory portion of the nasal flow trace . Scoring of FL events was manually performed⁵³.

Scoring of EEG

Sleep stages were scored according to the AASM rules¹⁴. The definitions of EEG activation and EEG arousal were based on both the CAP rules²⁴ and the AASM criteria¹⁴.

The CAP measures and EEG arousals were visually detected and automatically quantified with the RemLogic™PSG Software (Embla Systems).

The following CAP variables were measured: CAP time (CAP time in NREM sleep), CAP rate (the percentage ratio of CAP time to NREM sleep time) in total NREM sleep, and the percentage of each phase A subtype (A1,A2, A3).

The sleep parameters analyzed in our patients were compared with those of normal age- balanced subjects (controls), selected from the database of the Parma University Sleep Disorders Center.

Scoring of PWA

As already described in previous studies, the PWA drops were used as a indicator of finger vasoconstriction⁴⁸. In this study, only PWA drops $\geq 30\%$ were scored as clear signs of autonomic activation^{49,50}(Fig I-1).

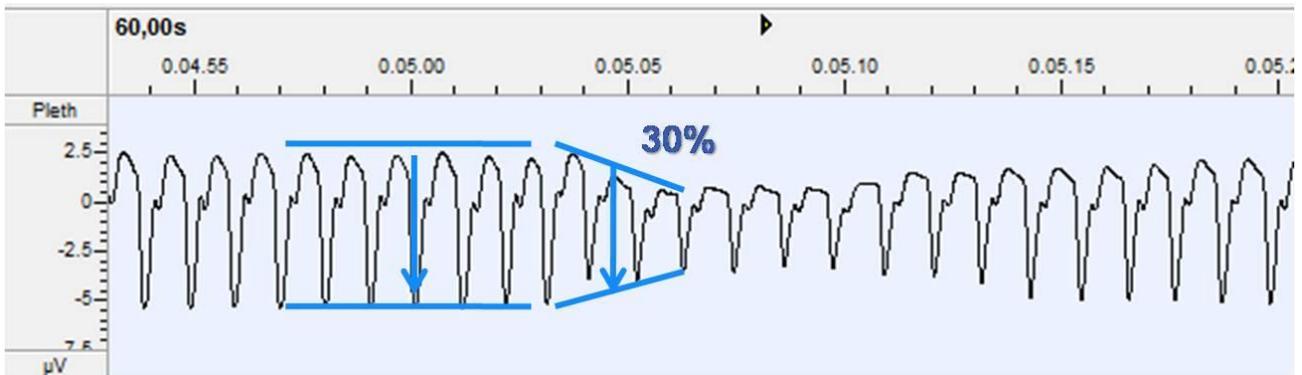


Figure I- 1: A significant PWA drop

Abbreviations. Pleth: finger photoplethysmogram.

Temporal relation between arousal responses (cerebral and autonomic) and respiratory events

Analysis of cerebral and autonomic response was focused in a time range of 4 seconds before and 4 seconds after respiratory recovery ¹⁰ (Fig I-2).

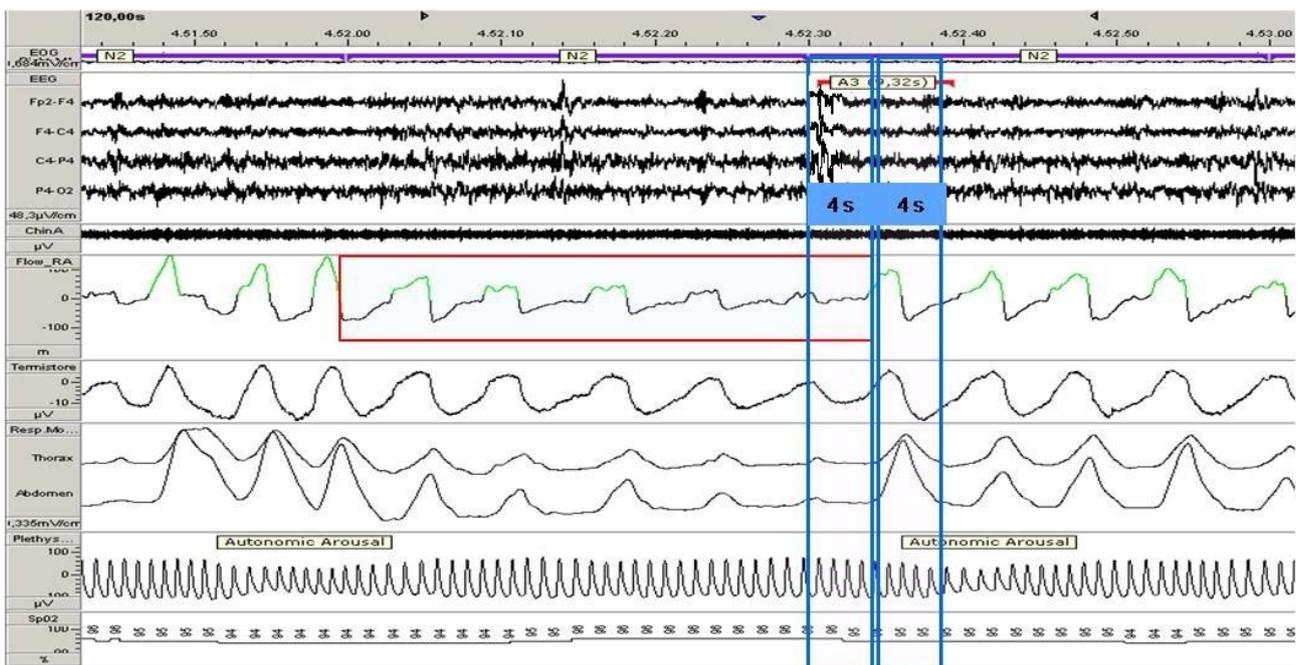


Figure I- 2: Temporal relation between respiratory recovery and arousal response

In the red box a FL event is showed; in the blue boxes are showed a time range of 4 seconds before and 4 seconds after respiratory recovery.

Abbreviations. EOG: electrooculographic channels, EEG: electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer, Termistore: oronasal thermal sensor, Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, Plethys: finger photoplethysmogram, SpO2: oxygen saturation.

The respiratory events (AP, H, FL) with a CAP A phase occurring in this time interval were considered associated with CAP (Fig I-3).

If there was no CAP A phase, but a PWA drop occurred, the AP, H or FL was scored as a respiratory event with autonomic response (Fig I-4).

The respiratory events that were not associated to an A phase or to a PWA drop were classified as isolated (Fig I-4).

An additional scoring was used for the FL events to detect the presence of AASM arousals in the same time interval before and after respiratory recovery: when FL led to an AASM EEG arousal the event was scored as RERA (Fig I-3).

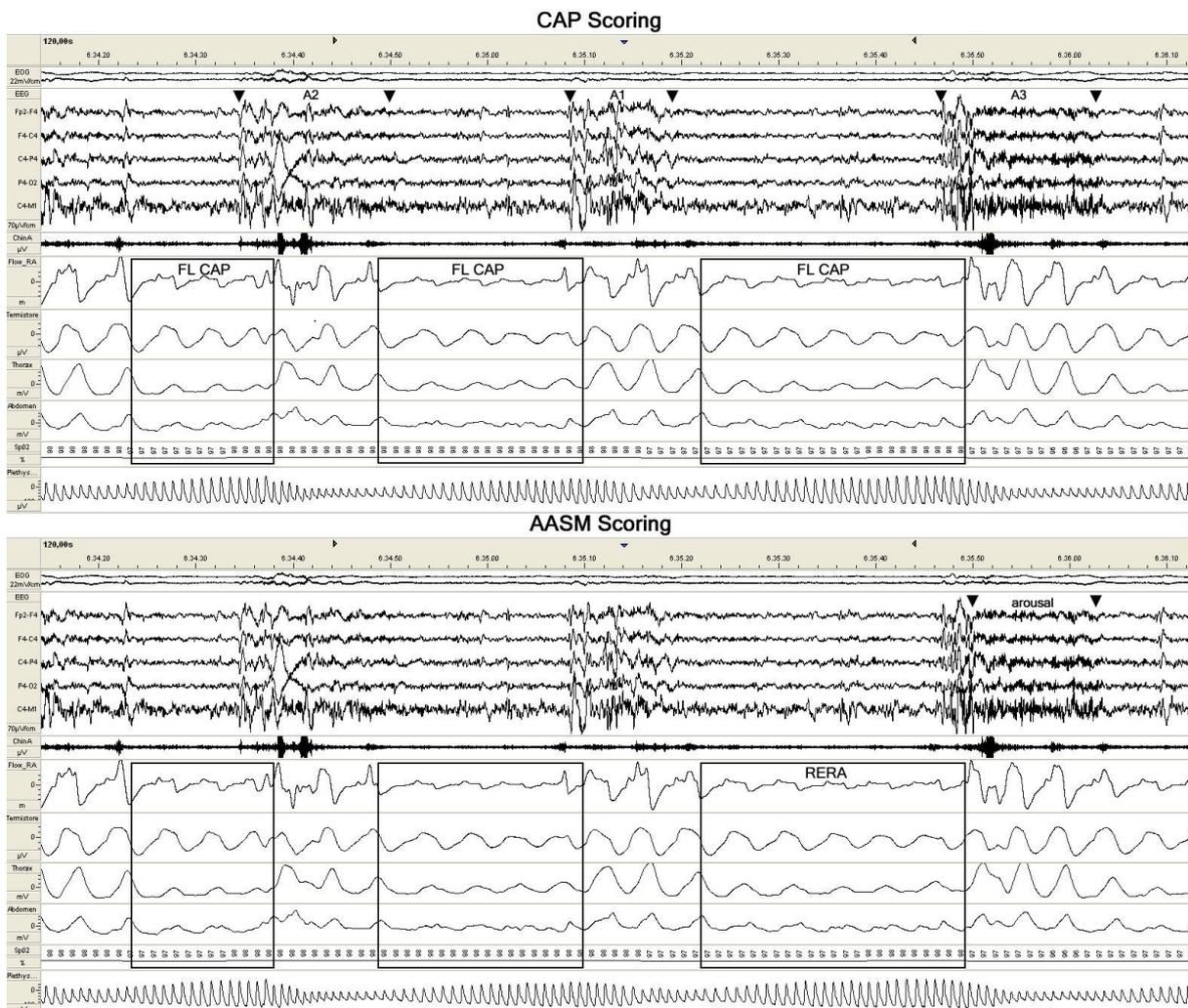


Figure I- 3: Arousal responses to the respiratory events

The flow limitation events are showed in the box. Flow limitation events associated with the A phase are presented in the top part of the picture, where the definition of EEG activation is based on the CAP rules. In the bottom part, the same sleep epochs in which the arousal is scored based on the AASM criteria, only a RERA is detected.

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

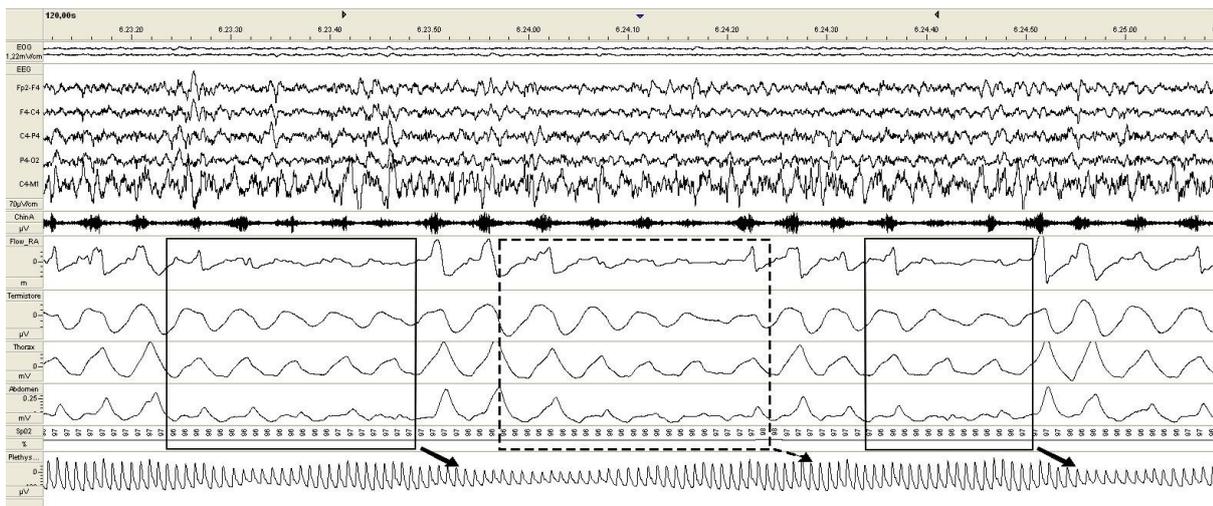


Figure I- 4: Respiratory events without EEG activation.

In the boxes are showed flow limitation events with autonomic response (PWA drops). An isolated flow limitation is presented in the dashed box.

Abbreviations. EOG; electrooculographic channels, EEG;electroencephalographic traces, Chin A; submental electromyogram, Flow RA; nasal air pressure transducer , Termistore; oronasal thermal sensor,Thorax; toracic effort by respiratory inductive plethysmograph, Abdomen; abdominal effort by respiratory inductive plethysmograph, SpO2; oxygen saturation, Plethys; finger photoplethysmogram.

Statistical analysis

Analysis was performed during Total Sleep Time (TST), NREM sleep and rapid eye movement (REM) sleep. Data are presented using descriptive statistics, between group difference comparisons were performed using a non-parametric ANOVA test (Kruskal-Wallis) and Mann-Whitney test. The level for statistical significance was established at $p < 0.05$. All statistical analyses were performed using IBM SPSS statistic 19.

Results

The selected PSG recordings showed high variability of the AHI (AHI range: 5-85), with an extensive distribution covering all severity levels of the OSAS pathology (Fig I-5).

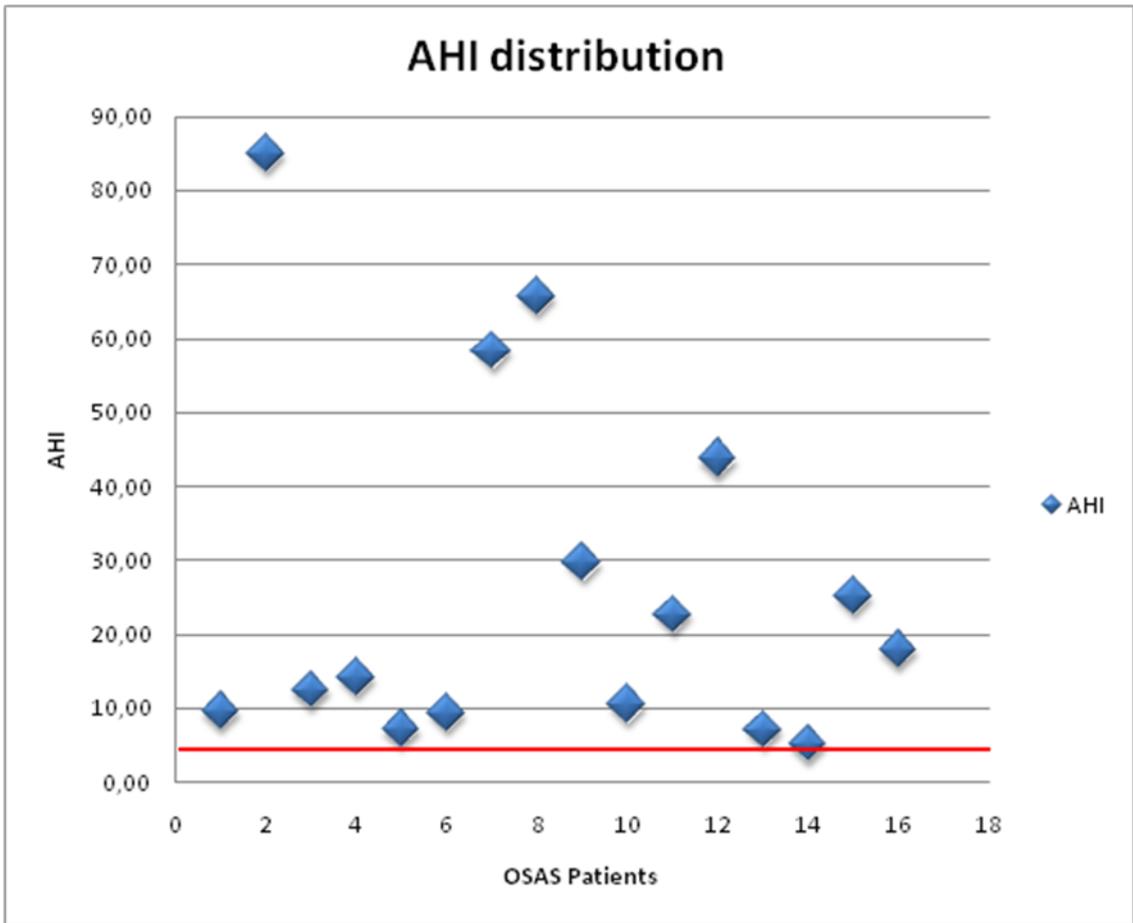


Figure I- 5: AHI distribution in OSAS patients

The mild group was composed of 8 patients, while the moderate-severe group included 11 patients. There were no age differences between two OSAS patients groups ($p=0.2818$).

Anthropometric, clinical and respiratory measures of the 19 patients are shown in Table I-1.

The PSG data of the normal subjects and the two groups of OSAS patients are summarized in Table I-2. No OSAS patient showed a periodic limb movement disorder.

| Characteristics | MS group | M group |
|---|-----------------|----------------|
| Age (years) | 51,91 (11,68) | 57,38 (8,88) |
| Body Mass Index (kg/m²) | 31,48 (7,99) | 25,04 (3,49) |
| Neck circumference (cm) | 44,88 (5,33) | 38,88 (2,10) |
| Mallampati (n) | 3,25 (0,71) | 3,57 (0,53) |
| Epworth Sleepiness Score (n) | 6,00 (2,98) | 8,13 (4,91) |
| Apnea Hypopnea Index (n/h) | 44,02 (21,09) | 8,33 (2,84) |
| Respiratory Disturbance Index (n/h) | 47,23 (18,82) | 15,76 (5,34) |
| Oxygen Desaturation Index (n/h) | 42,37 (21,62) | 7,00 (3,13) |
| Average SaO₂ (%) | 93,33 (2,70) | 95,45 (1,15) |
| Time SaO₂<90% (%) | 13,23 (19,27) | 0,28 (0,33) |
| Total Sleep Time Spent in Supine Body Position (%) | 40,05 (28,96) | 39,13 (26,99) |

Table I- 1. Clinical-anthropometric characteristics and polysomnographic respiratory pattern of two patient groups

Events are reported as mean value (SD) per night. Abbreviations. MS group: moderate-severe group, M group: mild group.

| Sleep parameters | N | M group | MS group | P-value |
|------------------------|--------------|---------------|----------------|----------------------|
| TST (min) | 457,1(24,75) | 417,56(46,33) | 445,35(77.20) | Ns |
| SE (%) | 88,74(7,14) | 80,15(14,92) | 84,71(11,60) | Ns |
| WASO (min) | 37,79(22,64) | 86,63(61,18) | 68,55 (47,76) | p 0.0180 N<M=MS |
| SL (min) | 10,50(15,98) | 6,75(4,31) | 10,50 (15,98) | Ns |
| NREM% | 75,64(2,98) | 83,91(17) | 87.17(7.98) | p 0.0001 N<M=MS |
| REM (%) | 25,19(2,93) | 16,09(8,09) | 12,83 (9,98) | p < 0.0001 N>M=MS |
| AI in NREM(n/h) | 16,44 (8.83) | 32,76 (12.95) | 44,66 (17.49) | p<0.001 N<M=MS |
| AI in REM (n/h) | 16.95 (8.30) | 37.38 (26.67) | 38.18 (26.82) | p<0.0382 N<M=MS |
| CAP time (min) | 152,5(20,76) | 180,64(34,76) | 282,27(58,02) | p < 0.0001 N<M<MS |
| CAP rate (%) | 45,65(7,71) | 52,20(10,32) | 73,95 (14,36) | p < 0.0001 N=M<MS |
| A1 (%) | 57,11(7,34) | 32,95(15,32) | 28,76 (15,32) | p < 0.0001 N>M=MS |
| A2 (%) | 29,16(4,30) | 11,33(3,00) | 13,53 (5,329) | p < 0.0001 N>M=MS |
| A3 (%) | 13,73(3,05) | 55,71 (4,07) | 57,71 (17,01) | p < 0.0001 N<M=MS |

Table I- 2: Comparison between normative subjects and OSAS patients for the sleep parameters.

Events are reported as mean value (SD) per night.

Abbreviations. TST :total Sleep Time, SE: Sleep Efficiency, WASO: wake time after sleep onset, SL: Sleep Latency, NREM%: percentage of time spent in non rapid eye movements sleep, REM% sleep :

percentage of time spent in rapid eye movements sleep, AI: arousal index, CAP time: total minutes of cycling alternating pattern, CAP rate(%):cycling alternating pattern rate, A1(%):percentage of CAP-phase A1, A2(%):percentage of CAP-phase A2, A3(%):percentage of CAP-phase A3, C : control group, MS group: moderate-severe group, M group: mild group.

P-values are from non-parametric test (Mann-Whitney): comparisons between the three groups (normative subjects(N), mild OSAS (M) and moderate-severe OSAS (MS) patients) were made (Mann-Whitney) when ANOVA reached significance (p value < 0.05).

Sleep Parameters

The PSG data of the conventional sleep parameters (macrostructure) and CAP variables of the controls and the two groups of OSAS patients are summarized in Table I-2.

Compared to normal subjects both OSAS groups showed an increase of WASO (wake after sleep onset) and NREM sleep percentage and a reduction of REM sleep percentage, (Tab I-2). The arousal index both in NREM and REM sleep was significantly increased in OSAS patients regardless of clinical severity. (Table I-2). No OSAS patient showed a periodic limb movement disorder.

The CAP time showed a progressive enhancement from normal subjects (152,5±20,76) to mild (180,64±34,76) and moderate-severe (282,27±58,02) OSAS patients. A significant increase of CAP rate was found only in the moderate-severe group. Both mild and moderate-severe OSAS patients showed the same distribution of the phase A subtypes of CAP with an increase of the A3 percentages and a reduction of A1 and A2 percentages compared to normal subjects.

Respiratory Events

A total of 5113 respiratory events were analyzed in NREM sleep: 1587 OA, 525 MA, 169 AC, 1181 H, 1651 FL. In REM sleep a total of 820 events were identified: 242 OA, 39 MA, 7 AC, 274 H, 258 FL.

The distribution of respiratory events in TST, NREM sleep and REM sleep were differently expressed in the two groups of OSAS patients (Tab I- 3).

As predictable, the moderate-severe group showed a higher number of AP and H, while in the mild group FL represented a more frequent event.

| Respiratory Events | TST | | NREM sleep | | REM sleep | |
|--------------------|----------|---------|------------|---------|-----------|---------|
| | MS group | M group | MS group | M group | MS group | M group |
| A(OA+MA) | 47,06 | 22,38 | 48,66 | 19,76 | 35,51 | 32,57 |
| H | 28,26 | 14,71 | 26,69 | 13,08 | 39,67 | 21,07 |
| FL. | 24,68 | 62,91 | 24,65 | 67,16 | 24,82 | 46,36 |
| FL. CAP | | | 18,56 | 50,84 | | |
| RERA | 8,71 | 31,38 | 8,66 | 33,24 | 9,06 | 24,14 |

Table I- 3: Distribution of respiratory events in sleep;

Events are reported as percentage of total respiratory events (AP, H and FL)

Abbreviations: TST :total Sleep Time , NREM sleep :non rapid eye movements sleep, REM sleep : rapid eye movements sleep, OA: obstructive apnea , AM: mixed apnea , H: hypopnea , FL: flow limitation, FL .CAP: flow limitation linked to CAP A –phases, RERA : respiratory effort related-arousal, MS group: moderate-severe group, M group: mild group.

When the two severity groups were compared for TST and NREM sleep, a significant difference was found in the amount of AP and H but not for FL events (Fig I-6,7). No statistical difference occurred in REM sleep (Fig I-8).

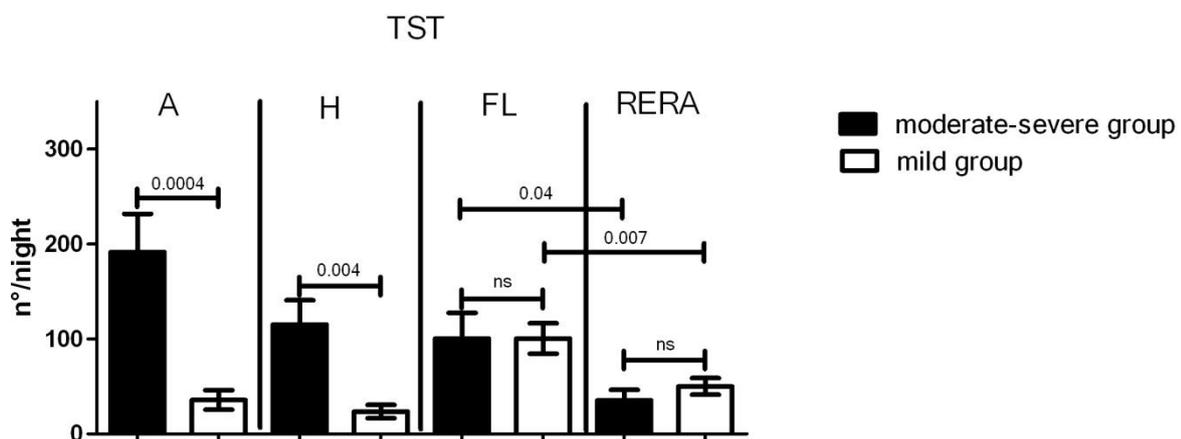


Figure I- 6: Distribution of respiratory events in the two groups of patients in total sleep time.

Events are reported as mean value \pm standard error of the mean (SEM) per night. Statistical analysis by non-parametric test (Mann-Whitney) between the 2 group are reported.

Abbreviations TST :total Sleep Time , A: obstructive apnea plus mixed apnea , H: hypopnea , FL: total flow limitation , RERA : flow limitation event linked to AASM arousal.

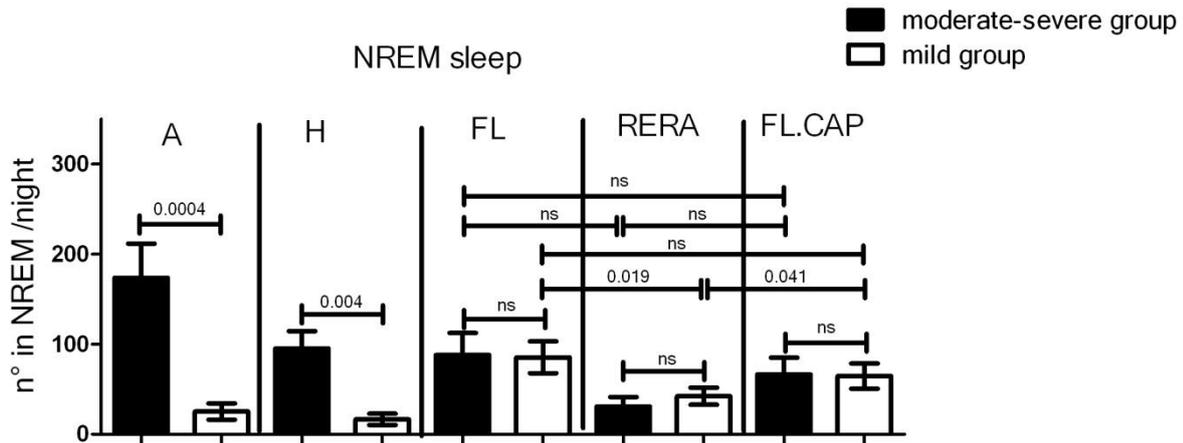


Figure I- 7: Distribution of respiratory events in the two groups of patients in non rapid eye movements sleep.

Events are reported as mean value \pm standard error of the mean (SEM) per night. Non-parametric analysis (Mann-Whitney) between the 2 group are reported.

Abbreviations NREM sleep :non rapid eye movements sleep, A: obstructive apnea plus AM:mixed apnea , H: hypopnea ,FL: total flow limitation , FL .CAP: flow limitation linked to CAP A -phases, RERA : flow limitation linked to AASM arousal.

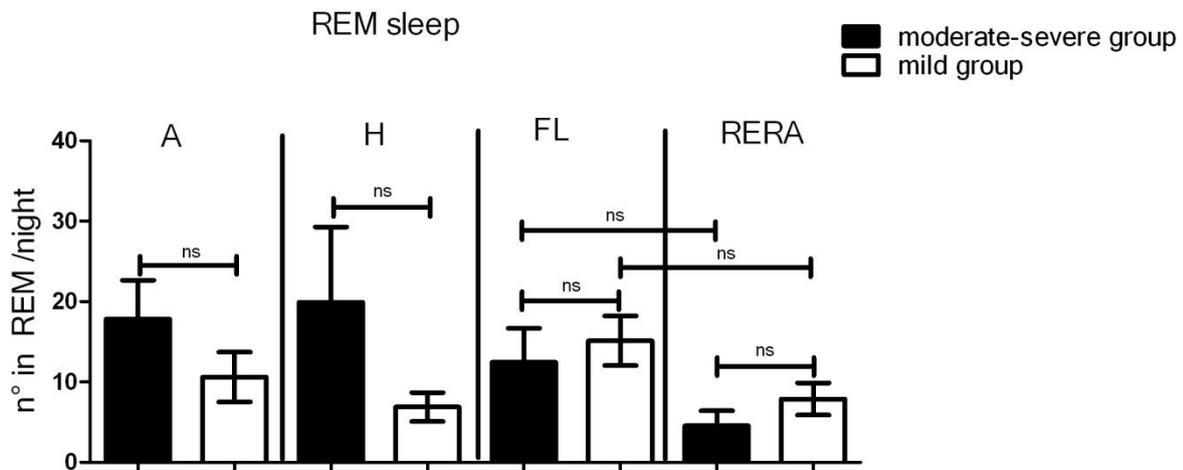


Figure I- 8: Distribution of respiratory events in the two groups of patients in rapid eye movements sleep.

Events are reported as mean value \pm standard error of the mean (SEM) per night. Non-parametric analysis (Mann-Whitney) between the 2 group are reported.

Abbreviations. NREM sleep :non rapid eye movements sleep, A: obstructive apnea plus AM: mixed apnea , H: hypopnea , FL: total flow limitation , FL .CAP: flow limitation linked to CAP A -phases, RERA : flow limitation linked to AASM arousal.

In NREM sleep, the analysis of CAP associated with respiratory events showed 1465 A phases related to OA, 503 A phases related to MA, 1026 A phases related to H and 1246 A phases related

to FL. In most cases, (75.6% of OA, 95 % of MA, 66.5% of H, 69.1% of FL), respiratory events ended with the CAP phase A3 subtype (Tab I-4).

| MS group | | | M group | | | |
|-----------|-------------|-------------|-------------|-----------|-----------|-------------|
| | A1 | A2 | A3 | A1 | A2 | A3 |
| OA | 14.1 (19.9) | 15.1 (14.2) | 89.4 (47.8) | 1.2 (2) | 3.0 (4.9) | 15.5 (15.3) |
| MA | 1.1 (2.9) | 1.0 (1.8) | 42 (90.8) | 0.1 (0.3) | 0.0 (0.0) | 1.8 (2.4) |
| H | 16.5 (20.1) | 14.8 (12.4) | 50.6 (35.1) | 0.6 (0.9) | 1.6 (1.7) | 13.2 (16.2) |
| FL | 13.4 (18.6) | 10.1 (8.4) | 42.6 (50.6) | 7.3 (8.5) | 8.2 (8.0) | 49.0 (30.1) |

Table I- 4: Links of respiratory events with A- phases of CAP in NREM sleep

Events are reported as mean value (SD) per night.

Abbreviations: OA: obstructive apnea ,AM:mixed apnea , H: hypopnea , FL: flow limitation, MS group: moderate-severe group, M group: mild group.

The respiratory events with only an autonomic response were 309 in NREM sleep (6.2 % of all respiratory events in NREM sleep) and 188 in REM sleep (23.1% of all respiratory events in REM sleep).

The isolated events were 395 in NREM sleep (8% of respiratory events in NREM) and 147 in REM sleep (18% of respiratory events in REM). No statistical differences were found between the two groups for autonomic and isolated events.

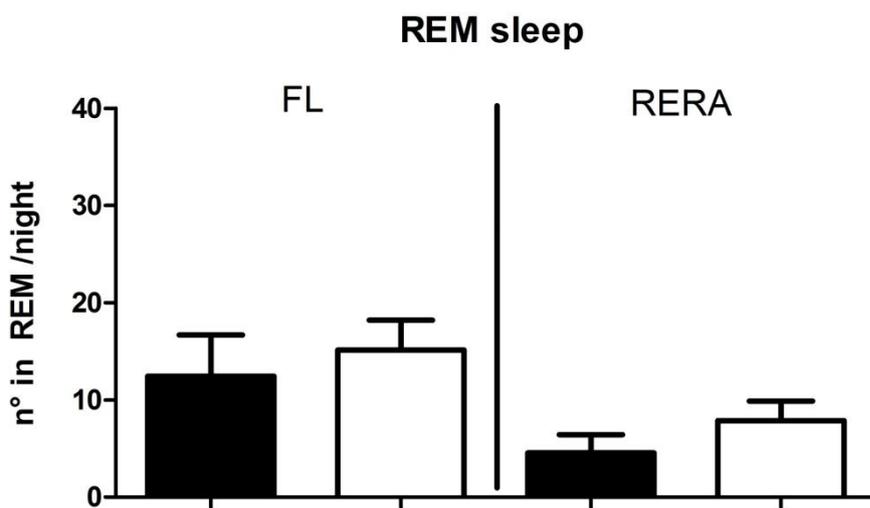
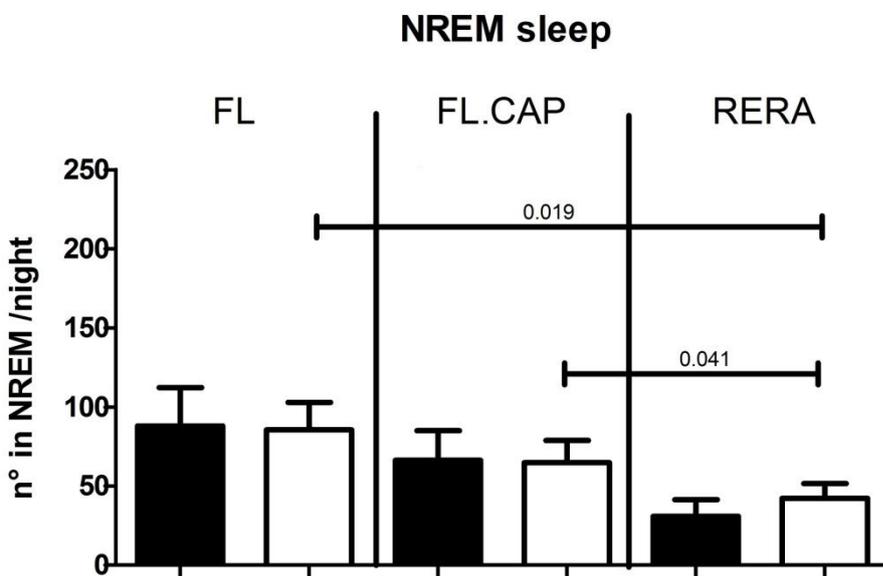
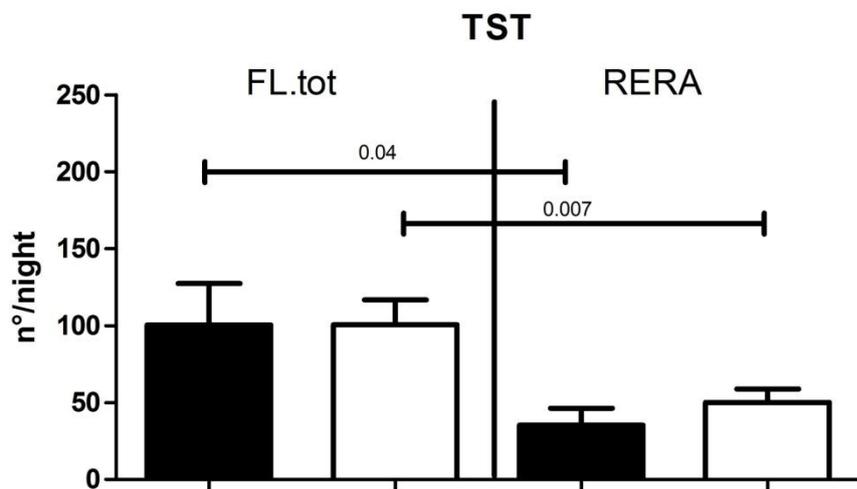
When we analyzed the FL events, we showed:

FLCAP: in NREM sleep, 1246 FL were associated to A phases , without significant difference between the two severity group (Fig. I-9). 861 of FLCAP ended with the A3 CAP phase and 385 with A1 and A2 CAP phases.

RERA: FL events scored as RERA were 678 in NREM sleep, and 113 in REM sleep. No significant difference was found between the two OSAS patients group (Fig I-9).

Non-EEG correlated FL: non-EEG correlated FL were 406 in NREM and 145 in REM sleep. No statistical differences were found between the two groups.

The comparison among FL, FLCAP and RERA is also shown in Figure I-9. In both OSAS severity groups, FL events were significantly higher than RERA in TST but not in REM sleep. In NREM sleep, FL events and FLCAP were significantly higher than RERA only for mild OSAS.



moderate-severe group
 mild group

Figure I- 9: 4 Distribution of FL in the two groups of patients in total sleep time, NREM and REM sleep.

Events are reported as mean value \pm standard error of the mean (SEM) per night. Statistical analysis by non-parametric test (Mann-Whitney) between the 2 groups are reported.

Abbreviations TST: total sleep time ; NREM sleep :non rapid eye movements sleep; NREM sleep: non rapid eye movements sleep; A: obstructive apnea plus mixed apnea ; H: hypopnea ; FL: total flow limitation ; RERA: flow limitation event linked to AASM arousal; FL CAP: flow limitation linked to CAP A –phases

Discussion

Sleep parameters

Compared to normal subjects, both OSAS groups showed a significant fragmentation of sleep (high amount of WASO) and alteration of sleep macrostructure (decrement in REM sleep and an increase in NREM sleep). While the arousal index was statistically similar in the two OSAS groups, sleep instability, expressed by CAP time, showed a progressive enhancement from normal subjects to mild and moderate-severe OSAS patients. The moderate severe group showed a significant increase of CAP rate and A3 phases, while a normal CAP rate coexisted with a higher amount of A3 subtypes in the mild group.

These results suggest that, under the thrust of breathing impairment (from mild to moderate severe OSAS), the sleeping brain exploits the available arousal responses⁵⁴, shifting the arousal response from the weaker A1 phases towards the more powerful A3 subtypes to interrupt the respiratory event and recover effective breathing.

The predominance of A3 subtypes even in patients in which CAP rate is similar to normal subjects, could be an early marker of sleep fragmentation in OSAS. These data are in agreement with the evidence that the sleeping brain is a complex system that modulates its internal states according to a continuous elaboration of the ongoing inputs⁵⁵. The CAP variables and the traditional PSG parameters represent the gradual adaptive solutions not only in the environment-sleep relationship⁵⁶, but also in the regulation of internal homeostasis⁵⁷.

Through this wide range of reactivity, the sleeping brain selects the different responses that put the system in a flexible balance between sleep maintenance and respiratory functionality. Similar to the rising levels of acoustic perturbation⁵⁶, the increase of OSAS severity determines progressive sleep instability and structural alterations as reflected by CAP parameters and conventional sleep measures.

Cerebral and autonomic responses to the respiratory events

The severity of OSAS affected also the features of respiratory events: compared to AP and H, FL events dominated in the mild group where they represented the prevalent respiratory pattern.

These findings suggest that as OSAS severity increases these subtle respiratory events evolve into major respiratory events such as AP and H. A similar progression has been described from UARS (upper airway resistance syndrome) to OSAS⁵⁸. In the recent years, the literature has demonstrated that there are different phenotypes of OSAS⁵⁹ that may vary over time, with aging, weight shifts, and other events including sleep deprivation, changes in autonomic activation, exposure to intermittent hypoxia. Therefore, OSAS breathing abnormalities during sleep include a spectrum of obstructive events from simple snoring to FL, RERA, H and AP⁶⁰. Accordingly, the International Classification of Sleep Disorders⁶¹ recommends that UARS should be included as part of OSAS and not considered as a separate entity.

In limited groups of patients Tamisier et al.⁶² showed a prevalent RERA pattern in UARS, an obstructive hypopnea-apnea pattern in severe OSAS and a mixed pattern in moderate OSAS. As an integrative contribution, our data found a prevalent FL pattern in mild OSAS, suggesting a possible underestimation of this features in non severe OSAS when we base the PSG evaluation exclusively on the AHI. Therefore, appropriate tools of assessment are deemed necessary⁶⁰.

RERA scoring has proven to be a more sensitive index than AHI^{13,63}

According to number of studies, the arousal scoring in OSAS patients is considered very important^{10 11-13} and is crucial for the analysis of FL events^{10,12,13}. However, at the moment, a shared interpretation of EEG responses to the FL events is lacking. In our patients, only 41.4% of FL events analyzed in TST met the AASM criteria for the RERA definition (41,1% in NREM and 43,8% in REM), without any statistical difference between two groups.

Although this underestimation is present both in TST and in NREM sleep, when we analyze the REM sleep, the number of FL is not statistically different from RERA. This trend could be due to the low number of FL in REM, but also to the limited EEG responses available during REM sleep. As previously demonstrated, in OSAS patients the cerebral reaction, when present in REM sleep, consists exclusively of rapid EEG activity, i.e., AASM arousal or A3 of CAP^{15,25}.

The definition of arousal with the CAP rules provide more information than AASM criteria for the evaluation of cerebral impact of respiratory events (+54% in the moderate severe group; +35% mild group). In NREM sleep, 75.5% of FL events presented a CAP A phase at the end of the respiratory event.

These data suggest that the RERA scoring underestimates the disruptive influences of OSAS on sleep and the definition of arousal with the CAP rules provides more information than AASM criteria for the evaluation of cerebral impact of FL events in NREM sleep

As previously demonstrated for AP and H, most FL events (69,1%) terminated with a CAP phase A3 subtype, independent of the severity group. Nevertheless, also the slow components of CAP (A1 and A2 CAP A phases) were well represented (24% OA, 33% H; 30,9% FL), confirming spectral EEG analysis studies showing an increase of theta-delta power at the end of AP and H events in the absence of AASM arousal^{15,31,34,64}.

Analyzing the single patients, we observed a high variability in the CAP A phases response. Independently of the OSAS severity, some patients showed a pattern of EEG reaction to respiratory events dominated by the slow component of CAP (A1 phases). In other patients even milder respiratory events were associated with AASM arousal.

In the future it will be interesting to investigate these different OSAS phenotypes for better understanding which variables of sleep, arousal threshold, and respiratory response could affect the different patterns.

Most of the respiratory events that did not result in an EEG response were FL (18 % in TST). 8.6% of these events, without an associated CAP A phase, presented an associated autonomic arousal while 9.4% were isolated. In TST 28.8 % of FL were not associated with EEG change (24.5% in NREM and in 56.2% in REM) , and the majority (51.4% of the non EEG correlated FL) showed signs of autonomic arousal (Pulse Wave Amplitude drops and increase of heart rate).;

As described in the literature, arousals which are defined exclusively by autonomic changes, such as a transient increase in blood pressure or heart rate, may have a significant impact on daytime sleepiness in OSAS patients⁶⁵. were not evaluated, as most of our patients did not complain of excessive sleepiness (Tab I-1) and the heart rate response was not measured in detail. In the future it will be necessary to collect a more larger number of patients, to clarify the biological price of FL events during sleep in OSAS.

Limitations

Although the high number of respiratory events allowed us to collect an extensive sample of data, a limitation of the study is a small group of OSAS patients.

Based on our previous results⁴⁹, we chose a 30% drop in PWA as a cutting point to detect arousals or respiratory events, and contrary to Tauman et al.⁴², we did not define a duration of this drop because we chose simple criteria easily applicable in clinical practice. Further studies will show whether a better definition of PWA change may improve the clinical usefulness of this parameter.

Conclusions

Sleep macrostructure allows discriminating between control subjects and OSAS patients but only the CAP measures differentiate the severity of OSAS.

In this study we confirmed that the relationship between the respiratory events and the cerebral response is more complex than that suggested by the international classification of arousal in sleep

⁶⁶.

This can determine an incomplete clinical assessment, mostly in the mild group of OSAS patients, and a possible incorrect interpretation of the efficacy of ventilation therapy, particularly in patients with residual sleepiness.

These diagnostic and therapeutic problems can become even more critical when we use PM as an alternative approach to PSG.

In the estimation of cerebral impact of respiratory events, the CAP scoring parameters in sleep can offer more extensive information compared to the AASM rules.

In the future a revision of the definition of arousal in sleep including both the EEG parameters and autonomic variables should be taken into consideration.

Section II

AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF PULSE WAVE AMPLITUDE

Section II: AROUSAL RESPONSES TO RESPIRATORY EVENTS: THE ROLE OF PULSE WAVE AMPLITUDE

Background

In recent years it has become apparent that increases in upper airway resistance that do not cause complete pharyngeal airway collapse are as clinically important as apneas in terms of producing sleep fragmentation, daytime symptoms⁶⁷ and increased cardiovascular risk associated with sleep-disordered breathing^{68,69}.

This obstructive respiratory alteration may result in obstructive hypopneas, where there is a significant reduction in airflow or in flow limitation (FL) events or respiratory events related arousal (RERA), where there is high inspiratory resistance without a significant fall in airflow or arterial oxygen saturation⁷⁰. These subtle events can be characterized by three different polygraphic features: a variable reduction of flow, an increase in respiratory effort, and the occurrence of a microarousal ending the respiratory event⁷⁰. The obstructive nonapneic episodes (H,FL, RERA) are much more difficult to detect and classify than apneas unless sensitive measures of respiratory effort, airflow and arousal are employed. Indeed if such measures are not used it may be impossible to differentiate central from obstructive hypopneas, and FL and RERA may be missed⁷⁰.

This has led the American Academy of Sleep Medicine (AASM) Task Force⁶³ to affirm that the definition of a nonapneic events, such as hypopneas (a reduction rather than absence in airflow), continues to be an area of considerable controversy^{52,71-74} and the different hypopnea definitions can result in considerably different AHI values⁷⁴⁻⁷⁶.

Moreover the scoring of nonapneic episodes is characterized by significant interobserver variations, particularly when the reduction of airflow is small (mean reduction around 30%) and the increase in respiratory effort is limited⁷⁰.

The PSG is the gold standard for detecting these respiratory alterations but it is time-consuming, expensive and not always available.

In the last years the use of portable monitoring (PM) is becoming an increasingly accepted cost-effective alternative approach¹⁻⁶ but suffers from two major drawbacks: the lack of a reliable signal of inspiratory effort to discriminate between obstructive and central hypopneas, and a reliable surrogate marker of arousals resulting in underestimation of nonapneic episodes, compared to PSG⁷⁷.

Oesophageal pressure (Pes) is the “gold standard” for quantifying variations in intrathoracic pressure resulting from respiratory efforts. However, measuring Pes is invasive, often uncomfortable for the patient, interfering with sleep quality⁷⁸ and pharyngeal airway dynamics⁷⁹ and it is not easy applicable in the PM.

For these reasons in the recent years a number of studies has been focused on individuation of surrogate markers of arousals and inspiratory effort related to respiratory events .

Pulse transit time (PTT) has been proposed as an alternate means for quantifying respiratory effort by detecting changes in blood pressure oscillations associated with pleural pressure swings⁸⁰.

In a study aimed to compare Pes and PTT, for scoring of non apneic events, Argod et Al.⁷⁰ demonstrate that both PTT and Pes, when used as measures of respiratory effort, are effective at detecting non apneic events.

PTT has the advantage over Pes that it is also capable of detecting AASM arousal. All these results concerning the PTT have to be confirmed with different softwares and portable devices⁸¹.

PWA is a surrogate marker of AASM arousal⁶³ both in diagnostic studies⁸² for sleep disordered breathing and in studies under non invasive ventilation (NIV)⁷⁷.

In these studies PWA show a high sensitivity^{77,82} to detect EEG- arousal but a low specificity⁷⁷. A plausible explanation for this low specificity was suggested by Delessert et al.⁸²: PWA can be associated with subtle changes in cortical activity even in the absence of AASM arousals. Indeed the authors showed an increase in EEG power density in high frequency band such as alpha and beta (cortical arousal⁶³, A2 and A3 CAP A phases⁸³), but also in lower frequency, including theta and delta bands (subcortical arousal, A1 CAP A phases⁸³), during PWA drop.

Other markers of sympathetic nerve activity also exhibit the same properties. For instance, upper airway obstruction in OSAS patients is associated with reduced amplitude of peripheral arterial tonometry (PAT) signal also in the absence of detectable EEG arousal⁸⁴.

These results lead to suppose that the increased power density in low frequency bands associated with an autonomic reaction, such as a PWA drop, may represent a central nervous system mechanism to preserve sleep continuity⁸². This interpretation is possible only overcoming the classical definition of arousal as isolated low-voltage fast-rhythm EEG events, marker of sleep disruption⁶³. Therefore the concept of arousal must be extended, incorporating high-amplitude EEG bursts (A1 CAP a phases), a special kind of arousal process, mobilizing parallelly antiarousal swings⁸⁵.

Moreover as we have shown in the section I in the estimation of cerebral impact of respiratory events, the CAP scoring parameters in sleep can offer a more accurate detection of subtle respiratory events and sleep alterations in OSAS patients compared to the AASM rules.

The present study aims at assessing in OSAS patients whether PWA drops, associated with respiratory events, were correlated with changes in cortical activity, as measured by CAP parameters, both in apneic and in obstructive nonapneic episodes, and whether this autonomic marker could be considered as a surrogate marker of EEG arousals.

Materials

The PSG recordings of OSAS patients analyzed were the same described in the Section I

Methods

The PSG interpretation, carried out by two experienced sleep investigators (1 pulmonologist, 1 neurologist) was focused on the evaluation of the response (cerebral and autonomic) to respiratory events.

Only the events that met the agreement of both scorers were included in the data analysis.

The criteria applied for the scoring of respiratory events, EEG parameters and PWA drops were the same described in the Section I.

The definitions of EEG activation were based on the CAP rules²⁴ in non rapid eye movement (NREM) and on the AASM criteria⁶³ in rapid eye movement (REM) sleep. In REM sleep, owing to the lack of EEG synchronization, the A phases consist exclusively of desynchronized patterns (transient activation phases or microarousals). Under physiological conditions, the 4- to 5-min interval between these A phases⁸⁶ does not meet the temporal requirements for the scoring of CAP in REM sleep. Only, under extreme conditions of sleep fragmentation, characterized by a remarkable increase of EEG desynchronization, as occurs in severe OSAS, CAP sequences can be detected also in REM sleep. Because 8 of our patients had a mild OSAS, the CAP in REM was not detectable.

Evaluation of the response (cerebral and autonomic) to respiratory events

Analysis of cerebral and autonomic response was focused in a time range of 4 seconds before and 4 seconds after respiratory recovery¹⁰.

On the basis of presence/absence of EEG and autonomic arousal in this time interval the respiratory events were classified as:

-**Respiratory events (AP, H, FL) with dual response** (EEG-PWA response, **EEG-PWA-r**) in which both CAP A phase and PWA drop occurred after respiratory recovery;

- **Respiratory events (AP, H, FL) only with EEG response (EEG-r)** in which a CAP A phase occurred after respiratory recovery and there was no PWA drop;

-**Respiratory events (AP, H, FL) with autonomic response (PWA-r)** in which only a PWA drop occurred;

-Respiratory events that were not associated with an A phase or a PWA drop were classified as **not response (No-r)**

An additional scoring was used for the FL events to detect the presence of AASM arousals also in NREM sleep. This scoring has been useful to establish whether the PWA and CAP scoring are more accurate for the detection of subtle respiratory events compared to the AASM rules. FL events which led to an AASM EEG arousal met the criteria for the definition of RERA.

Statistical analysis

Analysis was performed during Total Sleep Time (TST), NREM sleep and REM sleep. Data are analyzed using a non-parametric ANOVA test (Kruskal-Wallis). A Mann-Whitney test was applied when ANOVA reached significance. The analysis of correlation were performed with Spearman's test. The level for statistical significance was established at $p < 0.05$. All statistical analyses were performed using IBM SPSS statistic 19.

Results

Anthropometric, clinical and respiratory measures of the 19 patients are shown in Table I-1.

The PSG data are summarized in Table I-2..

| Characteristics of OSAS Patients | |
|--|---------------|
| Age (years) | 54,21 (10,69) |
| Body Mass Index (kg/m ²) | 28,62 (7,04) |
| Neck circumference (cm) | 41,88 (4,99) |
| Mallampati (n) | 3,40 (0,63) |
| Epworth Sleepiness Score (n) | 6,94 (3,88) |
| Apnea Hypopnea Index (n/h) | 29,41 (23,81) |
| Respiratory Disturbance Index (n/h) | 36,95 (21,46) |
| Oxygen Desaturation Index (n/h) | 27,48 (24,20) |
| Average SaO ₂ (%) | 94,22 (2,55) |
| Time SaO ₂ <90% (%) | 7,77 (15,80) |
| Total Sleep Time Spent in Supine Body Position (%) | 39,64 (27,28) |

Table II- 1: Clinical-anthropometric characteristics and polysomnographic respiratory pattern of OSAS patients

Events are reported as mean value (SD).

| Sleep parameters | |
|------------------------|----------------|
| TST (min) | 433,65 (65,92) |
| SE (%) | 82,79(12,91) |
| WASO (min) | 76,16(51,51) |
| SL (min) | 8,92(12,36) |
| NREM% | 85,80(7,97) |
| REM (%) | 14,20(7,97) |
| AI in REM (n/h) | 37,84(26,01) |
| CAP rate (%) | 64,79(16,67) |
| A1 (%) | 30,53(14,75) |
| A2 (%) | 12,60(4,52) |
| A3 (%) | 56,87(15,45) |

Table II- 2: Sleep parameters in OSAS patients.

Events are reported as mean value (SD) per night.

Abbreviations. TST :total Sleep Time, SE: Sleep Efficiency, WASO: wake time after sleep onset, SL: Sleep Latency, NREM%: percentage of time spent in non rapid eye movements sleep, REM% sleep : percentage of time spent in rapid eye movements sleep, AI(n/h): arousal index, CAP rate(%):cycling alternating pattern rate, A1(%):percentage of CAP-phase A1, A2(%):percentage of CAP-phase A2, A3(%):percentage of CAP-phase A3

The distribution of respiratory events in TST, NREM and REM sleep is presented in the Table II-3.

| Events | TST | NREM | REM |
|---------------------|------|------|-----|
| AP (n) | 2391 | 2110 | 281 |
| H (n) | 1469 | 1198 | 271 |
| FL (n) | 1921 | 1665 | 256 |
| Total number | 5781 | 4973 | 808 |

Table II- 3:Distribution of respiratory events.

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, TST :total Sleep Time, NREM :non rapid eye movement, REM: rapid eye movement.

In TST, the analysis of cerebral and autonomic response to respiratory events showed that 71,83% of respiratory events were EEG-PWA-r, 10,34% were EEG-r, 9,49% were PWA-r and 10,08% were No-r (Table II-4).

EEG-PWA-r was the most frequent response for all subtypes of respiratory event (AP,H,FL) with a progressive reduction from AP to H and FL. Conversely the isolated response (EEG-r, PWA-r and No-r) showed a progressive increase from FL to H and AP (Table II-4).

87.7% of respiratory events with EEG activation showed also a PWA drop and 53,4% of the respiratory events without EEG activation presented a PWA drop.

| TST | | | | | |
|---------------|--------------|----------------|--------------|--------------|---------------|
| Event subtype | event number | EEG-PWA-r | EEG-r | PWA-r | No-r |
| AP | 2391 | 2033 (85.02 %) | 145 (6.06 %) | 120 (5.01%) | 88 (3.68 %) |
| H | 1469 | 1075 (73.17 %) | 130 (8.84 %) | 138 (9.39%) | 126 (8.57 %) |
| FL | 1921 | 1045 (54.39 %) | 323 (16.81%) | 286 (14.88%) | 369 (19.20 %) |
| tot. number | 5781 | 4153 (71.83 %) | 598 (10.34%) | 549 (9.49 %) | 583 (10.08 %) |

Table II- 4: Cerebral and autonomic response to respiratory events in TST

Events are reported as number (percentage of total respiratory events) in TST (AP, H and FL)

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, TST :total Sleep Time.

82,17% of respiratory events presented an EEG response, with or without PWA drop, at the respiratory recovery, while a PWA drop, with or without EEG response, was present in the 81,32% of the events.

When we analyzed the data as mean value\ night in TST the same distribution of response to respiratory events was showed (Figure II-1, Table II-5).

| TST | | | | | |
|-----------|------------------|-------------|-------------|-----------------|---|
| Event | EEG-PWA-r | EEG-r | PWA-r | No-r | Statistical tests (Kruskal Wallis, Mann Whitney) |
| AP | 107.0(28.07) | 7.63 (2.31) | 6.57(2.24) | 4.63(1.44) | Anova p<0.0001 EEG-PWA-r vs EEG-r .p<0.0001 EEG-PWA-r vs PWA-r.p<0.0001 EEG-PWA-r vs No-r: p<0.0001 EEG-r vs PAW-r: ns EEG.ans. vs No-r: ns PWA-r vs No-r: ns |
| H | 56.58 (12.75) | 6.84(2.99) | 7.26 (3.65) | 6.63 (3.28) | Anova p<0.0001 EEG-PWA-r vs EEG-r: p<0.0001 EEG-PWA-r vs PWA-r: p<0.0001 EEG-PWA-r vs No-r: p<0.0001 EEG-r vs PAW-r: ns EEG-r vs No-r: ns PWA-r vs No-r:ns |
| FL | 54.53 (8.30) | 17.00(5.16) | 15.05(3.66) | 14.05 (3.76) | Anova p<0.0010 EEG-PWA-r vs EEG-r: p0.0011 EEG-PWA-r vs PWA-r: p0.0006 EEG-PWA-r vs No-r: p0.0004 EEG-r vs PAW-r: ns EEG-r. vs No-r: ns PWA-r vs No-r: ns |

Table II- 5: Cerebral and autonomic response to respiratory events in TST

Events are reported as mean value ± standard error of the mean (SEM) per night

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, TST :total Sleep Time.

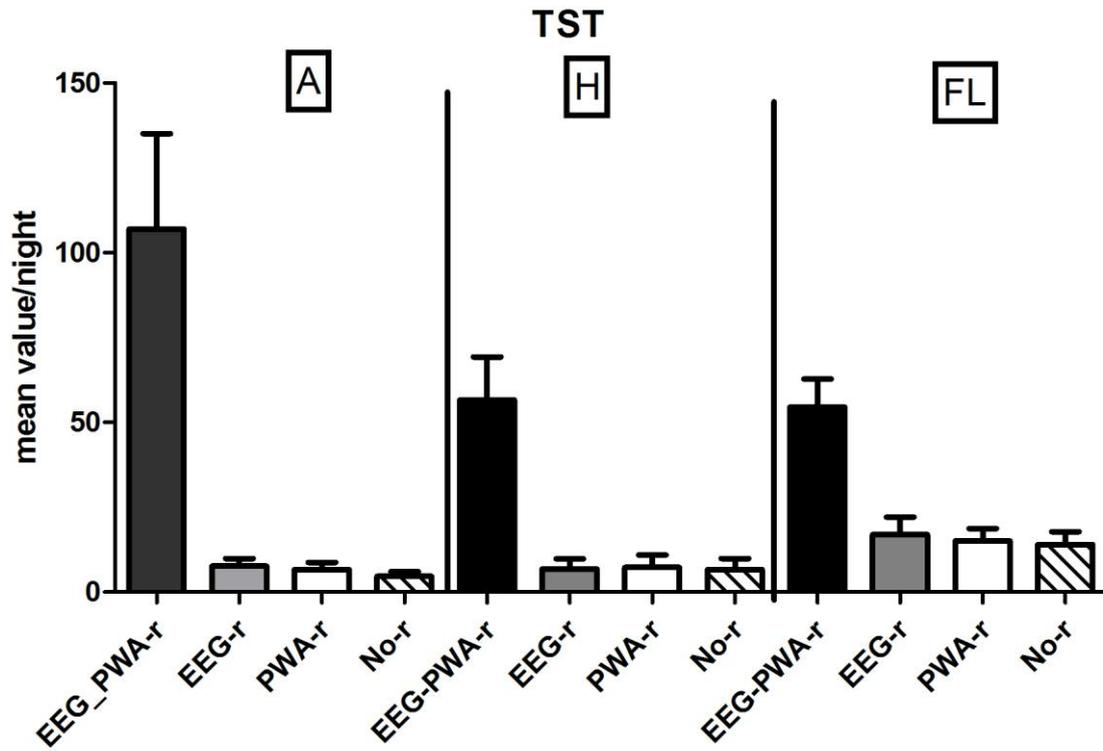


Figure II- 1: Cerebral and autonomic response to respiratory events in TST

Events are reported as mean value \pm standard error of the mean (SEM) per night

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, TST :total Sleep Time.

The correlation between events with EEG activation and events with PWA drops was high ($r = 0.9351$; $p < 0.0001$) (Fig II-1).

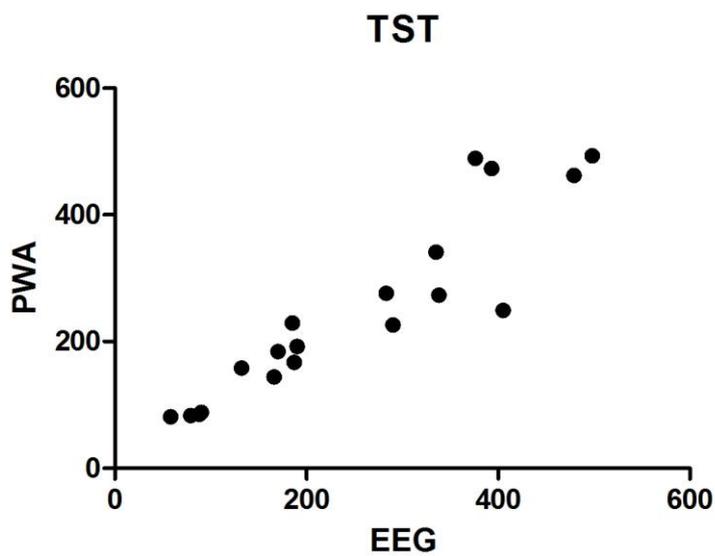


Figure II- 2: Correlation between respiratory events demonstrating an EEG activation, with or without PWA drop, and respiratory events with PWA drop (in presence or absence of EEG response) in TST.

Correlations were assessed by Spearman’s coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: TST: total sleep time, EEG-r: EEG response, PWA-r: PWA response.

Also in NREM sleep EEG-PWA-r was the most frequent response for all subtypes of respiratory event (AP,H,FL), with a progressive reduction from AP to H and FL. Conversely the isolated response (EEG-r, PWA-r and W-r) showed a progressive increase from AP to H and FL (Table II-6,Table II-7).

87,1% of respiratory events with CAP A phase showed also a PWA drop moreover 45,4% of the respiratory events without CAP response presented a PWA drop.

| NREM | | | | | |
|---------------------|---------------|----------------|---------------|---------------|---------------|
| Event | Events number | EEG-PWA-r | EEG-r | PWA-r | No-r |
| AP | 2110 | 1850 (87.67%) | 116 (5.49 %) | 87 (4.12 %) | 57 (2.70 %) |
| H | 1198 | 937 (78.21 %) | 119 (9.93 %) | 87 (7.26 %) | 55 (4.59 %) |
| FL | 1665 | 943 (56.63 %) | 312 (18.73 %) | 206 (12.37 %) | 204 (12.25 %) |
| Total number | 4973 | 3730 (75 .00%) | 547 (10.99 %) | 380 (7.64 %) | 316 (6.35 %) |

Table II- 6: Cerebral and autonomic response to respiratory events in NREM sleep

Events are reported as number (percentage of total respiratory events) in NREM (AP, H and FL)

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, NREM :non rapid eye movement.

| NREM | | | | | |
|-------|--------------|-------------|-------------|-------------|--|
| Event | EEG-PWA-r | EEG-r | PWA-r | No-r | Statistical tests (Kruskal Wallis, Mann Whitney) |
| AP | 97.37(26.46) | 6.10(2.04) | 4.57(1.86) | 3.00(1.21) | Anova $p < 0.0001$; Mann Whitney test : EEG-PWA-r vs EEG-r: $p < 0.0001$ EEG-PWA-r. vs PWA-r: $p < 0.0001$ EEG-PWA-r vs No-r: $p < 0.0001$ EEG-r vs PWA-r: ns EEG-r vs No-r: ns PWA-r vs No-r: ns |
| H | 49.16(11.53) | 6.26 (2.96) | 4.57(2.23) | 2.89 (1.18) | Anova $p < 0.0001$; Mann Whitney test : Mann Whitney test EEG-PWA-r vs EEG-r: $p < 0.0001$ EEG-PWA-r vs PWA-r.: $p < 0.0001$ EEG-PWA-r vs No-r: $p < 0.0001$ EEG-r vs PWA-r: ns EEG-r. vs No-r: ns PWA-r vs No-r: ns |
| FL | 49.16(7.84) | 16.42(5.13) | 10.84(3.02) | 10.74(3.01) | Anova $p < 0.0007$; Mann Whitney test : EEG-PWA-r vs EEG-r: $p = 0.0024$ EEG-PWA-r vs PWA-r: $p = 0.0003$ EEG-PWA-r vs No-r: $p = 0.0002$ EEG-r vs PWA-r: ns EEG-r. vs No-r: ns PWA-r vs No-r: ns |

Table II- 7: Cerebral and autonomic response to respiratory events in NREM sleep

Events are reported as mean value \pm standard error of the mean (SEM) per night

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, NREM: non rapid eye movement.

The correlation between EEG activation and PWA drops in response to respiratory events was high ($r = 0.9622$; $p < 0.0001$) (Fig II-3).

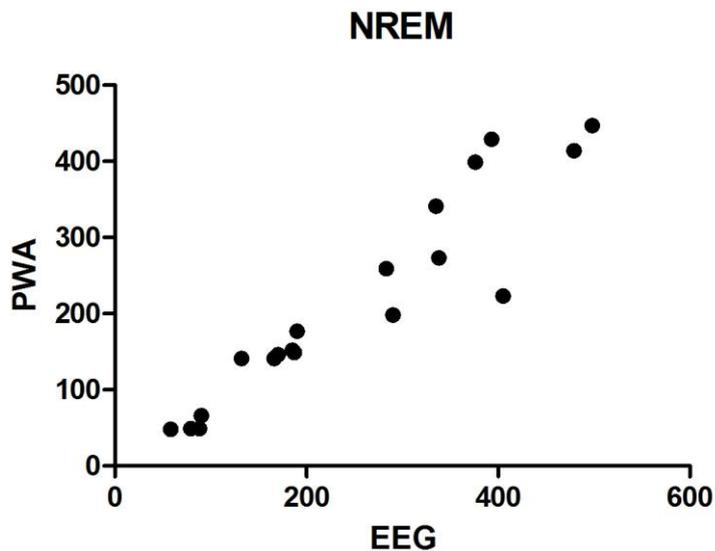


Figure II- 3: Correlation between respiratory events demonstrating a CAP A phase, with or without PWA drop, and respiratory events with PWA drop (in presence or absence of CAP A phase) in NREM sleep.

Correlations were assessed by Spearman's coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: NREM: non rapid eye movement, EEG-r: EEG response, PWA-r: PWA response.

In REM sleep the number of respiratory events was less than in NREM sleep, but the distribution of responses to respiratory events were the same (Table II-8, Table II-9).

89,3% of respiratory events with AASM arousal showed also a PWA drop moreover 53,4% (45,4% in NREM, 49,4% in REM) of the respiratory events without CAP response presented a PWA drop.

| REM | | | | | |
|---------------------|---------------|---------------|-------------|---------------|---------------|
| Event | Events number | EEG-PWA-r | EEG-r | PWA-r | No-r |
| AP | 281 | 183 (65.12%) | 29 (10.32%) | 38 (13.52%) | 31 (11.03%) |
| H | 271 | 138 (50.92 %) | 11 (4.05 %) | 51 (18.81 %) | 71 (26.19 %) |
| FL | 256 | 102 (54.39 %) | 11 (4.29 %) | 80 (31.25 %) | 63 (24.60 %) |
| Total number | 808 | 423 (52.35 %) | 51 (6.36 %) | 169 (20.91 %) | 165 (20.42 %) |

Table II- 8: Cerebral and autonomic response to respiratory events in REM sleep

Events are reported as number (percentage of total respiratory events) in REM (AP, H and FL)

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, REM : rapid eye movement.

| REM | | | | | |
|-------|------------|------------|------------|----------------|--|
| Event | EEG-PWA-r | EEG-r | PWA-r | No-r | Statistical tests(Kruskal Wallis, Mann Whitney) |
| AP | 9.63(2.48) | 1.52(0.69) | 2.00(0.77) | 1.63(0.67) | Anova p 0.0049; Mann Whitney test : EEG-PWA-r vs EEG-r: p0.0017 EEG-PWA-r vs PWA-r: p0.0025 EEG-PWA-r vs No-r: p0.0027 EEG-r:vs PWA-r: ns EEG-r vs No-r: ns PWA-r s vs No-r: ns |
| H | 7.26(2.55) | 0.57(0.24) | 2.68(1.52) | 3.73 (2.28) | Anova p 0.0157; Mann Whitney test : EEG-PWA-r vs EEG-r: p 0.0016 EEG-PWA-r vs PWA-r: p 0.0373 EEG-PWA-r vs No-r: ns EEG-r:vs PWA-r: ns EEG-r vs No-r: p0.0143 PWA-r vs No-r: ns |
| FL | 5.36(1.35) | 0.57(0.17) | 4.21(1.03) | 3.31(1.08) | Anova p 0.0019; Mann Whitney test : EEG-PWA-r vs EEG-r: p<0.0003 EEG-PWA-r vs PWA-r: ns EEG-PWA-r vs No-r: ns EEG-r vs PWA-r: p 0.0011 EEG-r vs No-r: p0.8824 PWA-r vs No-r : ns |

Table II- 9: Cerebral and autonomic response to respiratory events in REM sleep

Events are reported as mean value \pm standard error of the mean (SEM) per night

Abbreviations: AP: apnea , H: hypopnea , FL: flow limitation, EEG-PWA-r: EEG-PWA response, EEG-r:EEG response, PWA-r: PWA response, No-r: without response, REM: non rapid eye movement.

The correlation between EEG activation and PWA drops in response to respiratory events was moderate-high ($r = 0.7162$; $p = 0.0006$). (Fig II-4)

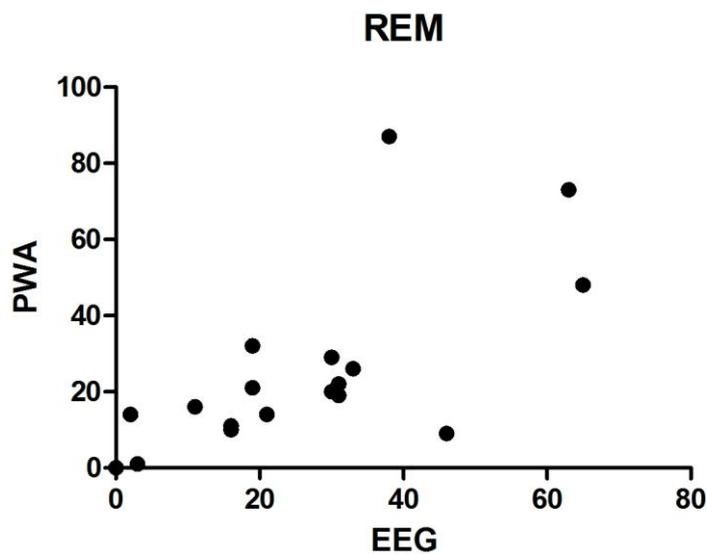


Figure II- 4: Correlation between respiratory events demonstrating an AASM arousal, with or without PWA drop, and respiratory events with PWA drop (in presence or absence of AASM arousal) in REM sleep.

Correlations were assessed by Spearman's coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: REM: rapid eye movement, EEG-r: EEG response, PWA-r: PWA response.

Additional scoring of the FL events with the AASM EEG arousal

In TST only 36,2% (35% in NREM, 44,1% in REM) of FL events showed an AASM EEG arousal at the respiration recovery and met the criteria for the RERA definition. The application of the AASM rules determined an underestimation of FL events not only compared to CAP scoring, as previous showed in the section I, but also compared to PWA scoring (Table II-10 and Figure II-5).

| | TST | NREM | REM |
|-----------------------------|--------------|--------------|------------|
| FL EEG-r (CAP rules) | | 65.58(12.06) | - |
| FL EEG-r (AASM rules, RERA) | 36.63(6.71) | 30.68(6.57) | 5.94(1.39) |
| FL PWA-r | 69.58(10.84) | 60.00(9.77) | 4.21(1.03) |
| FL No-r | 14.05(3.76) | 10.74(3.01) | 3.31(1.08) |

Table II- 10: Cerebral (CAP and AASM arousal) and autonomic response to FL events in TST, NREM e REM sleep

Events are reported as mean value \pm standard error of the mean (SEM) per night

Abbreviations: FL EEG -r (CAP rules): EEG response to FL, by means of CAP A phases, with or without PWA drop, FL EEG -r (AASM rules): EEG response to FL, by means of AASM arousal, with or without PWA drop, FL PWA-r: PWA response to FL with or without EEG response (CAP and AASM) , FL No-r: FL events without response, TST: total sleep time, NREM: non rapid eye movement, REM: non rapid eye movement.

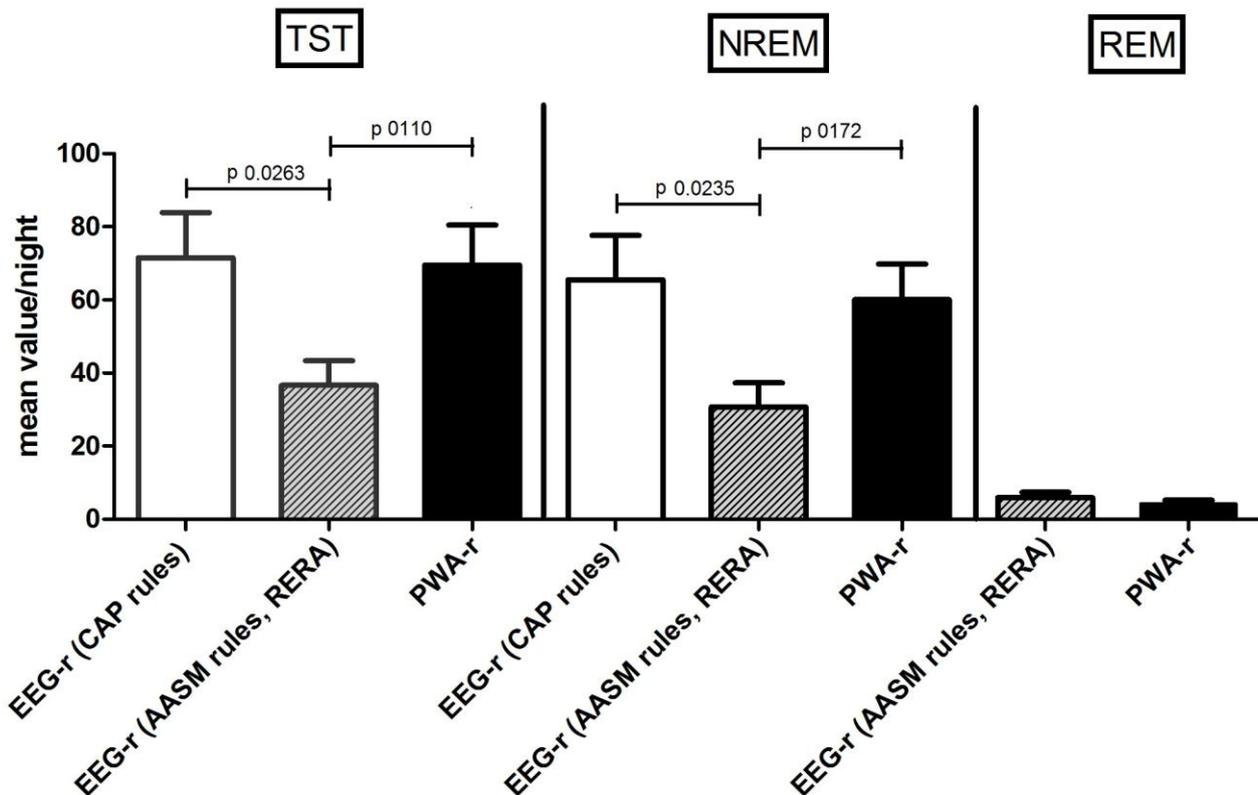


Figure II- 5: Distribution of cerebral (CAP and AASM arousal) and autonomic response to FL events in TST, NREM e REM sleep

Events are reported as mean value \pm standard error of the mean (SEM) per night. Statistical analysis by non-parametric test (Mann-Whitney) between the group are reported.

Abbreviations: EEG-r (CAP rules): EEG response to FL, by means of CAP A phases, with or without PWA drop, EEG-r (AASM rules, RERA): EEG response to FL, by means of AASM arousal, with or without PWA drop, PWA-r: PWA response to FL with or without EEG response (CAP and AASM), TST: total sleep time, NREM: non rapid eye movement, REM: non rapid eye movement.

The correlation between EEG activation, and PWA drops in response to respiratory events was high in TST (0,9178 $p < 0,0001$) and NREM sleep (0,9192 $p < 0,0001$) and moderated in REM sleep (0,4626 $p 0,0462$) (Fig II- 6-8).

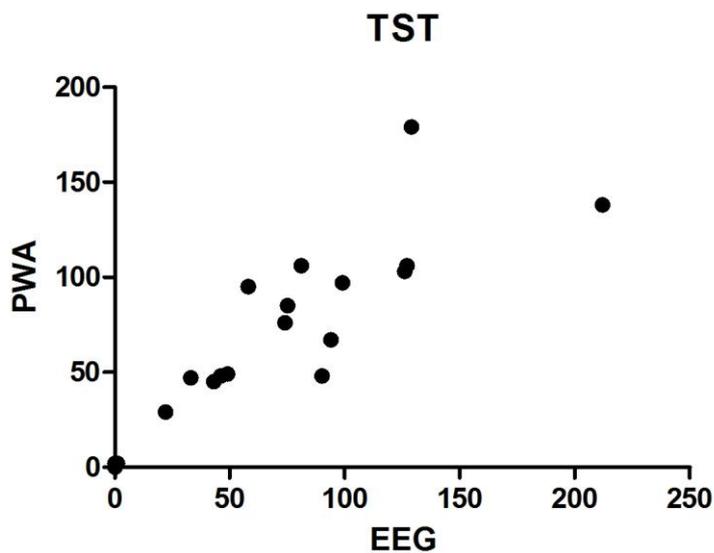


Figure II- 6: Correlation between FL events demonstrating an EEG response (CAP and/or AASM), with or without PWA drop, and respiratory events with PWA drop (in presence or absence of EEG response) in TST.

Correlations were assessed by Spearman's coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: TST: total sleep time, EEG-r: EEG response, PWA-r: PWA response.

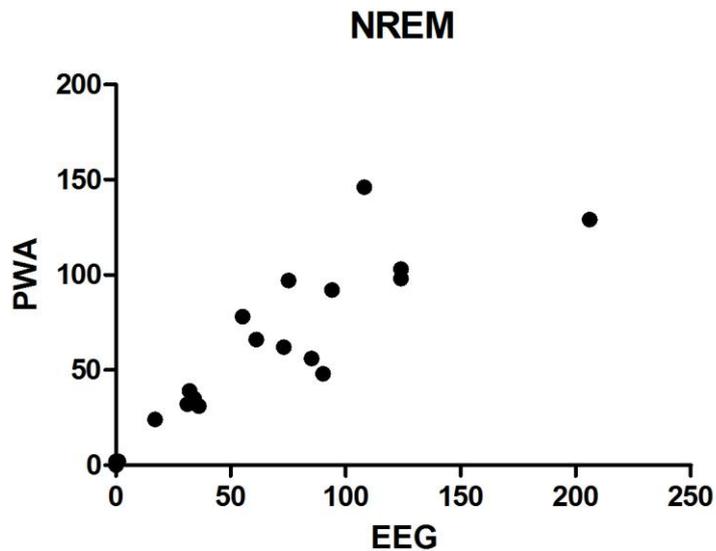


Figure II- 7: Correlation between FL events demonstrating an EEG response (CAP and/or AASM), with or without PWA drop, and respiratory events with PWA drop (in presence or absence of EEG response) in NREM sleep.

Correlations were assessed by Spearman’s coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: NREM: non rapid eye movement, EEG-r: EEG response, PWA-r: PWA response.

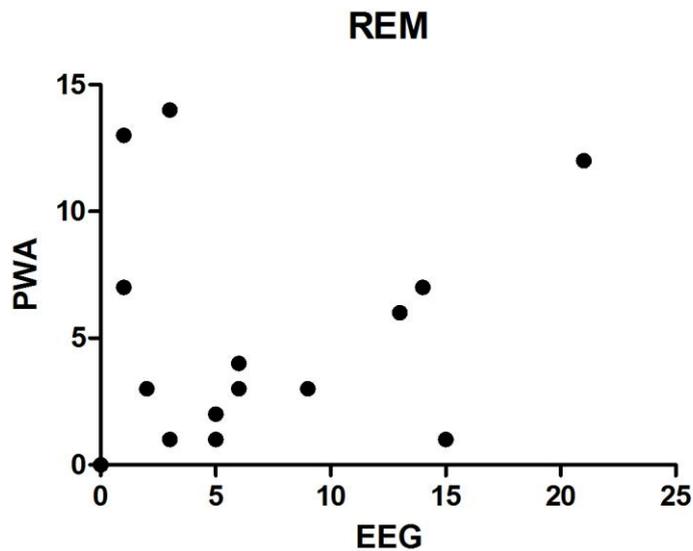


Figure II- 8: Correlation between FL events demonstrating an EEG response (AASM), with or without PWA drop, and respiratory events with PWA drop (in presence or absence of EEG response) in REM.

Correlations were assessed by Spearman’s coefficient.

Events are reported as mean value (SD) per night.

Abbreviations: REM: rapid eye movement, EEG-r: EEG response, PWA-r: PWA response.

Discussion

This study constitutes the first description of the relation between PWA drops and CAP A phases at the termination of respiratory events in OSAS patients.

Our data show that at the recovery of airway patency a dual response, with cortical and autonomic activation, is the most frequent manifestation.

As previously demonstrated, arousal in sleep is an essential element for homeostasis regulation, providing an excitation drive to vital processes⁸⁵. Arousal can be generated directly by the cortex under the impulse of the physiologic evolution of sleep or in response to a sensorial perturbation, such as respiratory interruption. In any case, the involvement of the brain makes the arousal a unitary phenomenon in which activation is modulated through a hierarchy.

The arousal is variable according to the different combinations of EEG, behavioral and autonomic activities. In spite of the differences in the intensity dimension and in the EEG, autonomic and behavioral components, the variable forms of arousals are supported by a uniform background along a hierarchic continuum^{87,88} concerning the degree of activation they produce.

When the arousal stimulus is of lower intensity or during slow wave sleep, the cortical areas might not be activated. In these cases the arousal response is expressed by delta and K-bursts (A1 phases) with or without autonomic activation. When the stimulus intensity is greater or when sleep is lighter, a delayed cortical activation is present, determining the transition from slow to fast desynchronized EEG activity (A2 and A3 phases), and a powerful activation of autonomic nervous system activity⁸⁵. The presence of a dual response at the termination of the respiratory events, showed in our patients, confirms that the brain under a severe perturbation, such as a reduction in airflow or an increase of upper airway resistance, induces, in most cases, a generalized activation of all subsystems. Moreover a hierarchy of response is confirmed by the progressive increase of the isolated responses (EEG-r, PWA-r and No-r) from the more relevant apneas to the flow limitation events. These findings suggest that when the obstruction of the airway increases, the arousal phenomena evolve into a more powerful activation. In the recent years, the literature has demonstrated that there are different phenotypes of OSAS⁵⁹ that may vary over time, with aging, weight shifts, and other events including sleep deprivation, changes in autonomic activation, exposure to intermittent hypoxia. Therefore, OSAS breathing abnormalities during sleep include a spectrum of obstructive events from simple snoring to FL, RERA, H and AP⁶⁰ that correlate with a hierarchic progression of arousal powerful.

The most important finding of our study is the significant correlation between CAP A phases (EEG activation) and PWA drops in response to respiratory events.

Previous findings demonstrated that PWA shows a good sensitivity^{77,82} to detect AASM arousals but a low specificity, because PWA drops can be also associated with subtle changes in cortical activity, as well as increase in EEG power density in lower frequency band, even in the absence of visually scored AASM arousals⁸². In the CAP framework, the cerebral response is a complex phenomenon in which the arousal definition is more extensive compared to the AASM criteria¹⁴, incorporating not only the low-voltage high-frequency EEG bands such as alpha and beta (AASM arousal or subtypes A2 and A3 of CAP A phases), but also the subtle changes characterized by an increase in the high-voltage lower frequency activities such as theta and delta bands (A1 CAP A phases). Our results allow us to state that in the estimation of cerebral impact of respiratory events, CAP scoring parameters and PWA analysis can provide more extensive information compared to the AASM arousal rules.

PWA can be easily derived from conventional pulse oxymeters, it lacks sleep alteration and can be analyzed online.

Conversely the scoring of AASM arousals, and the analysis of CAP parameters, are time-consuming and require a well-trained staff to achieve acceptable reliability.

For these reasons the use of PWA scoring as surrogate marker of cortical response to respiratory events may be an alternative option especially when the diagnosis of OSAS is based on PM devices.

Scoring of RERA requires the presence of AASM arousal⁶³ and because PM do not provide cortical arousal, the OSAS severity is underestimated when the simplified monitoring tools are compared to PSG. In our study, similarly to previous data⁸⁹, the application of PWA scoring increases the detection of subtle respiratory events, such as FL, compared to AASM rules. No statistical difference are showed between the number of FL associated with PWA drops and FL with CAP A phases in TST and NREM sleep. These data led us to say that the application of PWA scoring to PM could reduce the underestimation of OSAS severity.

Limitations

The 5781 respiratory events analyzed in the present study arise from a limited group of patients. Moreover different threshold values for PWA were not tested. Based on previous observations^{49,90}, we chose a 30% drop in PWA as a cutting point to detect arousals and respiratory events. It is possible that a lower threshold value would have increased sensitivity. However, spontaneous variations of PWA in the magnitude of 10-30% are reported in normal subjects reflecting physiologic oscillations in autonomic nervous system activity, and may have limited pathological significance.

Contrary to Tauman et al.⁴², we did not define a duration of this drop because we chose simple criteria easily applicable in clinical practice.

Further studies will show whether a better definition of PWA change may improve the clinical usefulness of this parameter.

Conclusions

The variable forms of arousals in OSAS patients are a hierarchic continuum^{36,87}, from isolated to dual response, that correlates with the severity of the respiratory events. In the estimation of the response to respiratory events, the CAP scoring and PWA analysis can offer more extensive information compared to the AASM rules.

Our data confirm also that the application of PWA scoring improves the detection of respiratory events and could reduce the underestimation of OSAS severity compared to AASM arousal.

In the future the introduction of an autonomic marker of arousal, such as PWA, will be topical to better evaluate the impact of the respiratory events on sleep.

The introduction of these parameters could reduce the gap between PSG and PM in the estimation of the OSAS severity.

CONCLUSIONS

Conclusions

The arousal scoring in Obstructive Sleep Apnea Syndrome (OSAS) is important to clarify the impact of the disease on sleep but the currently applied American Academy of Sleep Medicine (AASM) definition may underestimate the subtle alterations of sleep.

The relationship between the respiratory events and the arousal response is more complex than that suggested by the international classification. The variable forms of arousals in OSAS patients are a hierarchic continuum, from isolated to dual response, that correlates with the severity of the respiratory events. In the estimation of the response to respiratory events, the CAP scoring and PWA analysis can offer more extensive information compared to the AASM rules.

Our data confirm also that the application of PWA scoring improves the detection of respiratory events and could reduce the underestimation of OSAS severity compared to AASM arousal. These diagnostic and therapeutic problems can become even more critical when we use PM as an alternative approach to PSG.

In the future a revision of the definition of arousal in sleep including both the EEG parameters and autonomic variables should be taken into consideration and the introduction of autonomic marker of arousal , such as PWA, will be topical for better evaluate the impact of the respiratory events on sleep.

The introduction of these parameters could reduce the gap between PSG and PM in the estimation of the OSAS severity.

APPENDIX

Appendix: Introduction

CAP scoring rules

CAP is a well-defined marker of the physiological cerebral activity occurring under conditions of reduced vigilance (sleep, coma), translating a state of arousal instability and involving muscle, behavioral, and autonomic functions^{83,91,92}. During NREM sleep, CAP is organized in sequences. A CAP sequence is composed of a succession of CAP cycles. The CAP cycle is defined of an A phase (lumps of sleep phasic events) followed by a B phase (return to EEG background). All CAP sequences include at least two consecutive CAP cycles, and they always begin with a phase A and end with a phase B^{22,93}. Each phase, both A and B, is 2-60 seconds in duration (Fig A- 1).

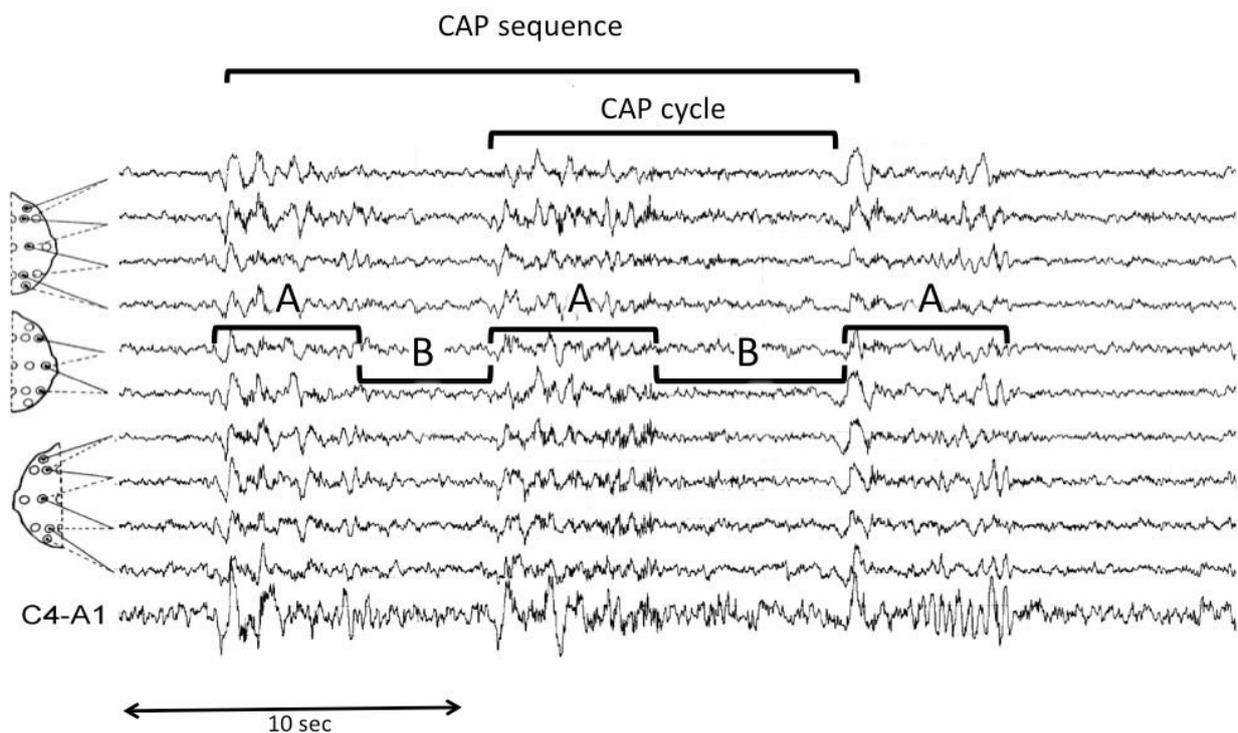


Figure A-1: Cyclic alternating pattern (CAP) during NREM sleep

A CAP cycle is defined as a sequence of 2 alternating stereotyped EEG patterns, each lasting more than 2 and less than 60 seconds, called A phase (A in the figure) and B phase (B). At least 2 full CAP cycles in succession are needed to define a CAP sequence; thus, the minimum content of a sequence is A-B-A-B-A. Bipolar EEG derivations using international electrode placement; from top to bottom: FP2-F4, F4-C4, C4-P4, P4-O2, F8-T4, T4-T6, FP1-F3, F3-C3, C3-P3, P3-O1 + monopolar derivation C4-A1. **Modified from Parrino et Al. (2010). Curr Opin Pulm Med 2014**

This cut-off relies on the consideration that the great majority (about 90%) of A phases occurring during sleep are separated by an interval of less than 60 seconds⁹⁴. The absence of CAP for more than 60 s is scored as non-CAP (NCAP) and coincides with a condition of sustained physiological stability⁹⁵. Isolated A phases are classified as NCAP. At least two consecutive CAP cycles are necessary to compose a CAP sequence. On the contrary, CAP sequences have no limit in duration or number of CAP cycles. The last A phase that closes a CAP sequence is not included in the scoring of CAP, as it is not followed by a corresponding B phase. Within NREM sleep, a CAP sequence is not interrupted by a sleep stage shift if CAP scoring requirements are satisfied. Consequently, because CAP sequences can extend across adjacent sleep stages, a CAP sequence can contain a variety of different phasic activities

In NREM sleep, the repetitive patterns of phase A are composed of the specific arousal-related phasic events peculiar to the single sleep stages:(a) intermittent [alpha] rhythms⁹⁶ and sequences of vertex sharp waves, in stage 1; (b) sequences of two or more k-complexes with⁹⁷ or without[alpha]-like components and [beta] rhythms, in stage 2; (c) [delta] bursts showing a difference in amplitude of at least one-third compared with background activities, in stages 3 and 4⁹⁸; (d) transient activation phases⁸⁶ or microarousals⁹⁹, in stages 1 and 2 or at the end of stages 3 and 4, characterized by increase of EEG frequency with decreased EEG amplitude, disappearance of sleep spindles and [delta] activities when occurring in SWS, transitory enhancement of muscle tone or appearance of electromyographic activities, body movements, and postural changes, and acceleration of heart rate⁸⁶.

In NREM sleep, the CAP sequences may extend across successive sleep stages, and thus the A phases may present different patterns within the same CAP sequence.

In REM sleep, owing to the lack of EEG synchronization, the A phases consist exclusively of desynchronized patterns (transient activation phases or microarousals). Under physiological conditions, the 4- to 5-min interval between these A phases⁸⁶ does not meet the temporal requirements for the scoring of CAP in REM sleep. However, under extreme conditions of sleep fragmentation characterized by a remarkable increase of EEG desynchronization, as occurs in OSAS, CAP sequences can be detected also in REM sleep.

In normal young adults, 2.5 min is the approximate mean duration of a CAP sequence, containing an average of 6 CAP cycles¹⁰⁰. Among the various CAP parameters, CAP rate is the most extensively used for clinical purposes: it is calculated as the percentage ratio of total CAP time to NREM sleep time¹⁰¹.

Recording techniques and montages

In all sleep stages, CAP appears as a synchronous and widely diffused EEG activity on both hemispheres with minor differences in morphology and amplitude across the various leads. Bipolar longitudinal montages such as Fp1-F3, F3-C3, C3-P3, P3-O1 or Fp2-F4, F4-C4, C4-P4, and P4-O2 warrant the most clear-cut detection of. A calibration of 50 mV/7 mm with a time constant of 0.1 s and a high-frequency filter in the 30 Hz range is recommended for EEG channels. Monopolar EEG derivations (C3-A2 or C4-A1 and O1-A2 or O2-A1), eye movement channels, and submentalis EMG, currently used for conventional sleep staging and arousal scoring, are also essential for CAP scoring²³.

CAP A phases subtypes

Phase A activities can be classified into three subtypes, referring to the reciprocal proportion of high-voltage slow waves (EEG synchrony) and low-amplitude fast rhythms (EEG desynchrony) throughout the entire A phase duration (Fig A 2) The three subtypes are described as follows:

- Subtype A1. EEG synchrony (high-amplitude slow waves) is the predominant activity. If present, EEG desynchrony (low-amplitude fast waves) occupies less than 20% of the entire phase duration.
- Subtype A2. The EEG activity is a mixture of slow and fast rhythms with 20% to 50% of phase A occupied by EEG desynchrony.
- Subtype A3. The EEG activity is dominated by rapid low-voltage rhythms with more than 50% of phase A occupied by EEG desynchrony.

A CAP sequence can include different phase A subtypes. The majority of AASM EEG arousals occurring in NREM sleep (87%) is inserted within CAP sequences and basically coincides with a phase A2 or A3^{83,102}. Among all subtypes, a hierarchical activation from the slower EEG patterns (moderate autonomic activation without sleep disruption) to the faster EEG patterns (robust autonomic activation associated with visible sleep fragmentation) has been described in different studies^{36,85,103–105}. If AASM arousal is a sign of transient sleep discontinuity, the finding of phasic EEG delta activities during enhancement of autonomic functions indicates the possibility of physiological activation without sleep disruption¹⁰⁶

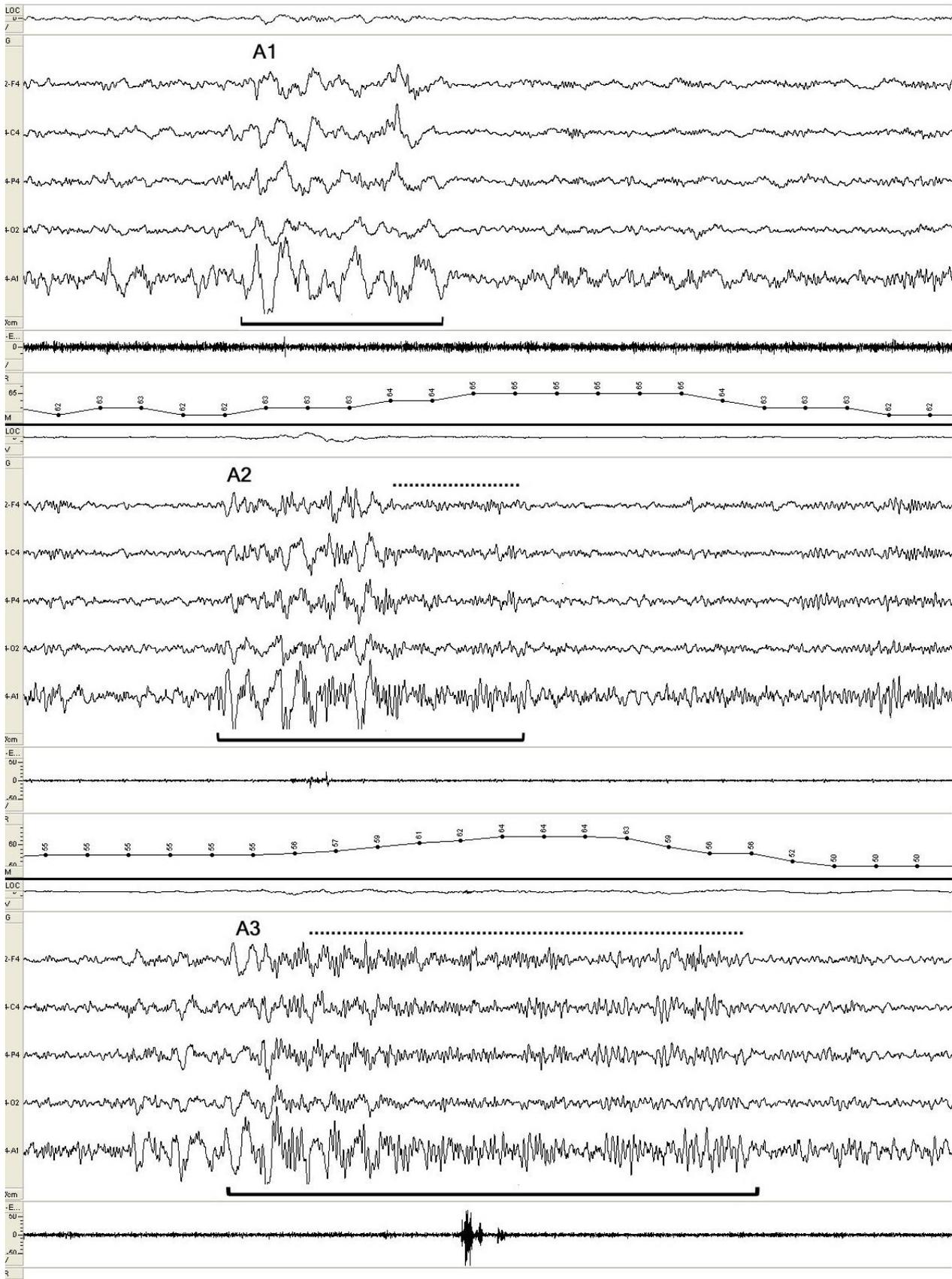


Figure A-2: CAP A-phases subtypes

The three phase A subtypes delimited by the black lines. From the top to the bottom: A1, A2, A3. The dotted spots indicate the fast low-amplitude portion of the A phase. Montage, from the top to the bottom: electrooculogram (ROCC-LOC); EEG Fp2-F4, F4-C4, C4-P4, P4-O2, C4-A1; chin electromyogram (EMG), heart rate (HR). **Modified from Parrino et Al. (2010). Curr Opin Pulm Med 2014 Nov;20(6):533-41**

PWA analysis

The skin sympathetic activity, mediated via postganglionic pathways, plays a major role in regulation of cutaneous blood flow¹⁰⁷. Beside the sympathetic nervous system output cutaneous finger blood flow is altered by systemic responses like changes in stroke volume, cardiac output, peripheral resistance, particularly in the arterioles, as well as by superimposed humoral and metabolic activation¹⁰⁸. Wall arteries are covered by muscles that contract or relax, which produces arterial constriction or dilatation. This process is regulated by several mechanisms, such as the vegetative system, which determines vascular muscle tone. The dominant system (i.e., sympathetic or parasympathetic) causes blood vessels to contract (vasoconstriction) or dilate (vasodilatation) The increase in sympathetic activity is associated with vasoconstriction and it is related to transient arousal¹⁰⁹

Pulse oximeters are used daily for arterial hemoglobin oxygen saturation (SpO₂) and pulse rate (PR) monitoring in various clinical settings. Conventional pulse oximetry uses two wavelengths of light (red and infrared) transmitted through the distal phalanx of the finger. Despite its simple appearance, the pulse oximeter photo plethysmographic waveform is a highly complex signal that contains more information than just PR¹¹⁰. The photo plethysmograph waveform is based on a signal proportional to infrared light absorption between an emitter and a photo detector, which are usually placed on the opposite sides of the fingertip (transmission photo plethysmography). The original raw signal is the current generated by the photo detector¹¹⁰ (Fig A-3).

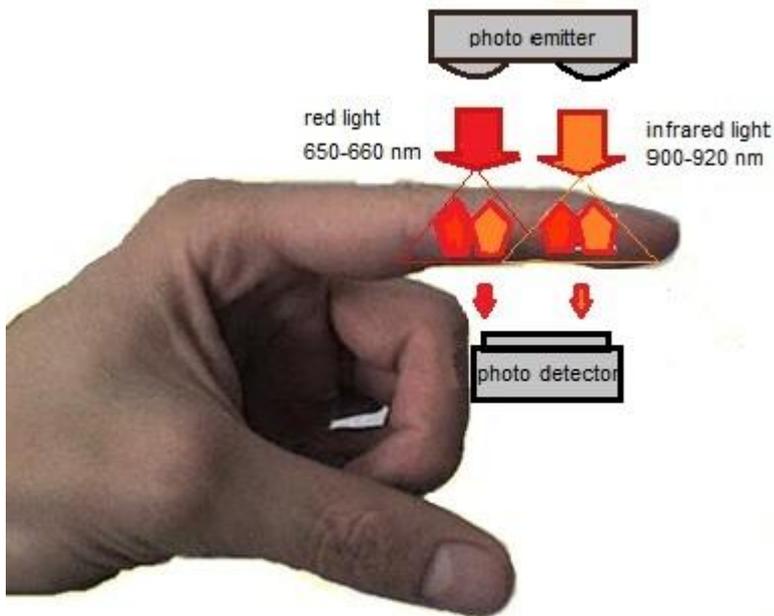


Figure A-3: A schematic representation of pulse oximeter photo plethysmograph

Pulse wave amplitude (PWA) is the most frequently used parameter obtained by finger plethysmography. PWA is directly and positively correlated to finger blood flow¹¹¹. Vasoconstriction is reflected in the PWA signal by decreases in the signal amplitude fluctuation (Fig A-4).

Pulse Wave Amplitude

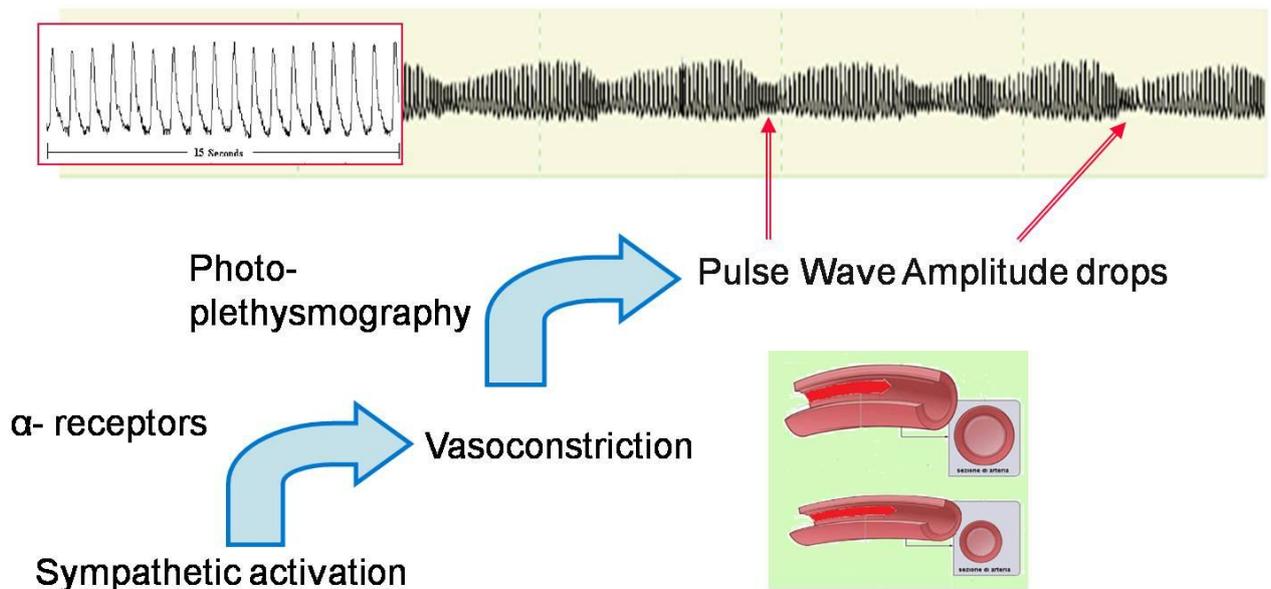


Figure A-4: A schematic representation of the PWA drops generation

Arterial blood flow pulsation passing through an artery modulates the light absorption and provokes a pulse wave easily convertible in an electrical signal readable during PSG. This parameter can be easily derived from conventional pulse oxymeters and does not bring any alteration of sleep. It has also been used as a marker of finger vasoconstriction⁵¹ and shown to be more sensitive than HR as a marker of autonomic and cortical arousal^{48,49}. Spontaneous variations of PWA in the magnitude of 10-30% are reported in normal subjects reflecting normal oscillations in autonomic nervous system activity, and may have no pathological significance, A 30% drop in PWA is a cutting point to detect arousals and respiratory events^{49,90}.

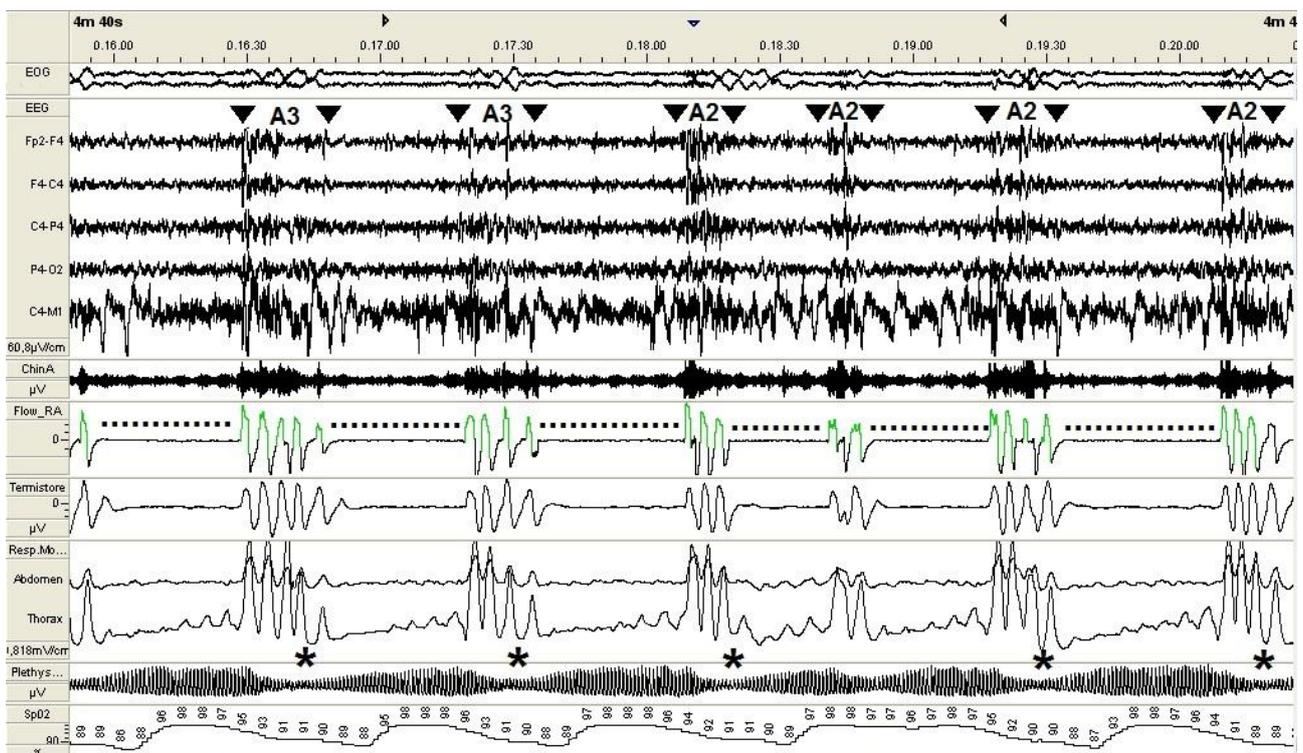


Figure A-5: Modulation of EEG and PWA response to respiratory events

Examples of respiratory events (dotted lines) in which airway re-opening occur with CAP a phases (delimited by black triangles) and PWA drops (asterisk).

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmograph.

Appendix: Section I

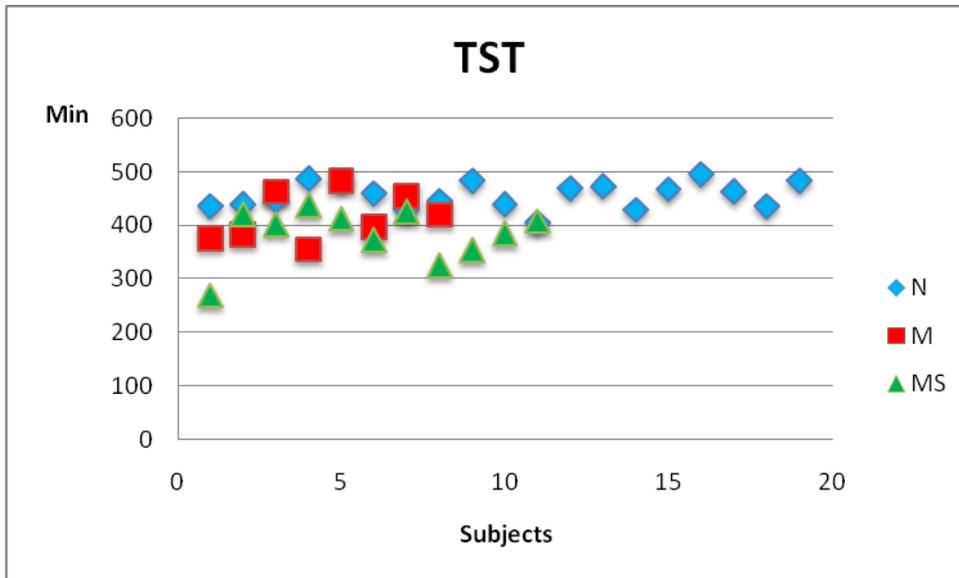


Figure A-6: Total sleep time (TST) in OSAS patients and normal subjects

Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

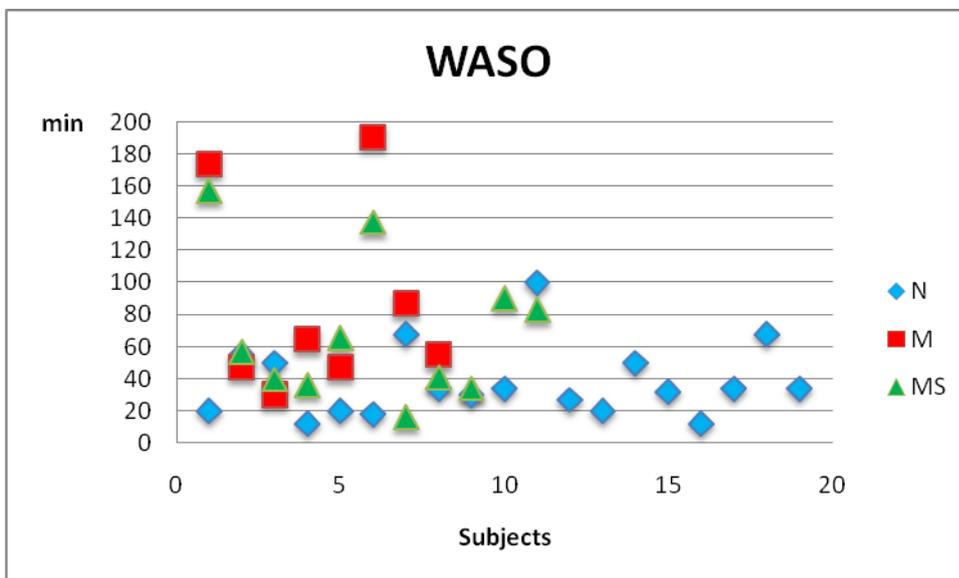


Figure A-7: Wake after sleep onset (WASO) in OSAS patients and normal subjects

Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

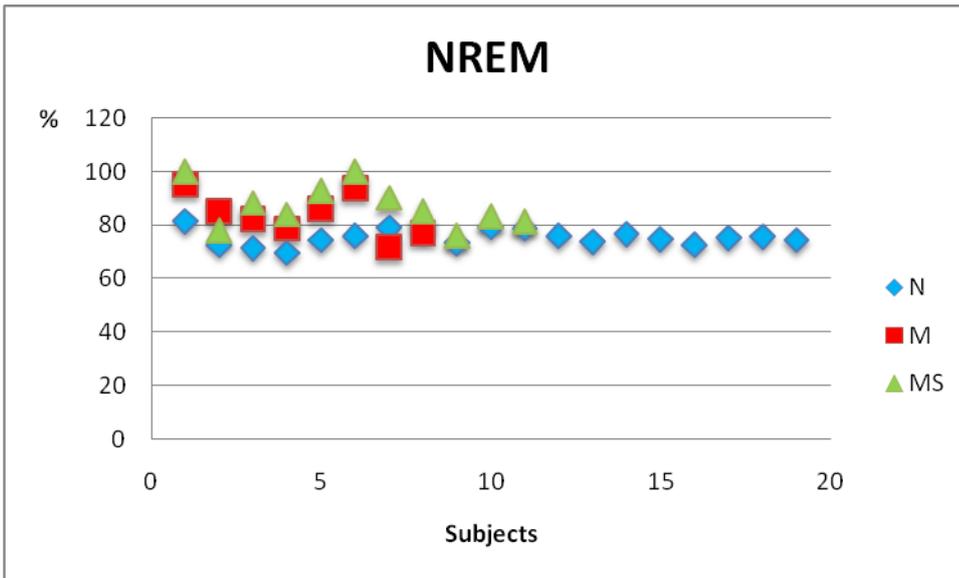


Figure A-8: Percentage of non rapid eye movement sleep (NREM) in OSAS patients and normal subjects
 Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

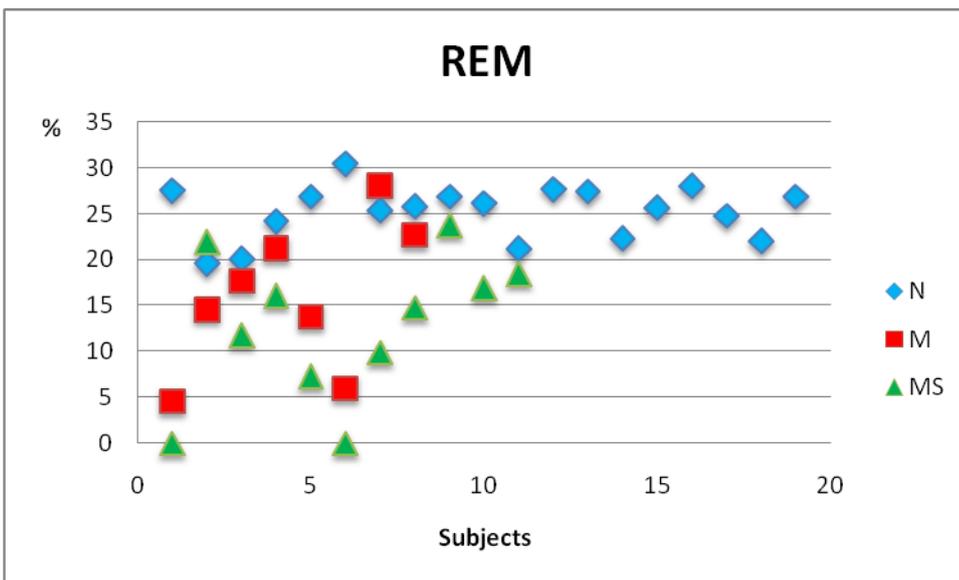


Figure A-9: Percentage of rapid eye movement sleep (REM) in OSAS patients and normal subjects
 Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

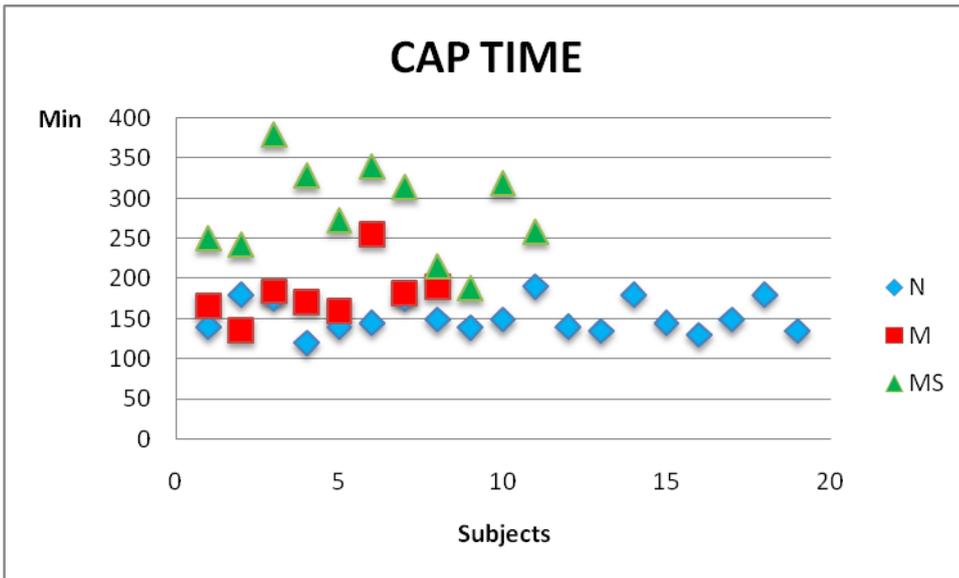


Figure A-10: Time of cyclic alternating pattern (CAP) in NREM sleep in OSAS patients and normal subjects
 Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

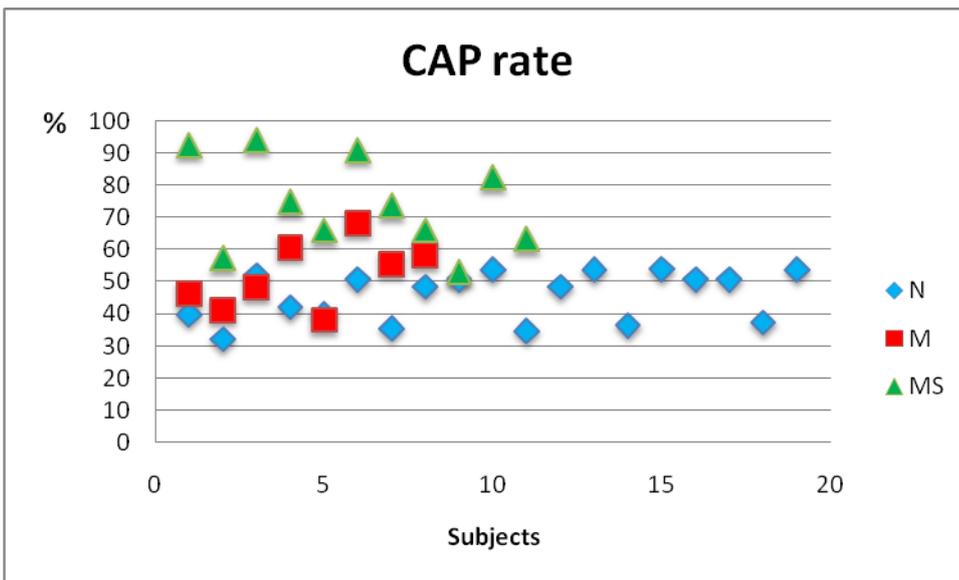


Figure A-11: Percentage of cyclic alternating pattern (CAP) in NREM sleep in OSAS patients and normal subjects
 Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

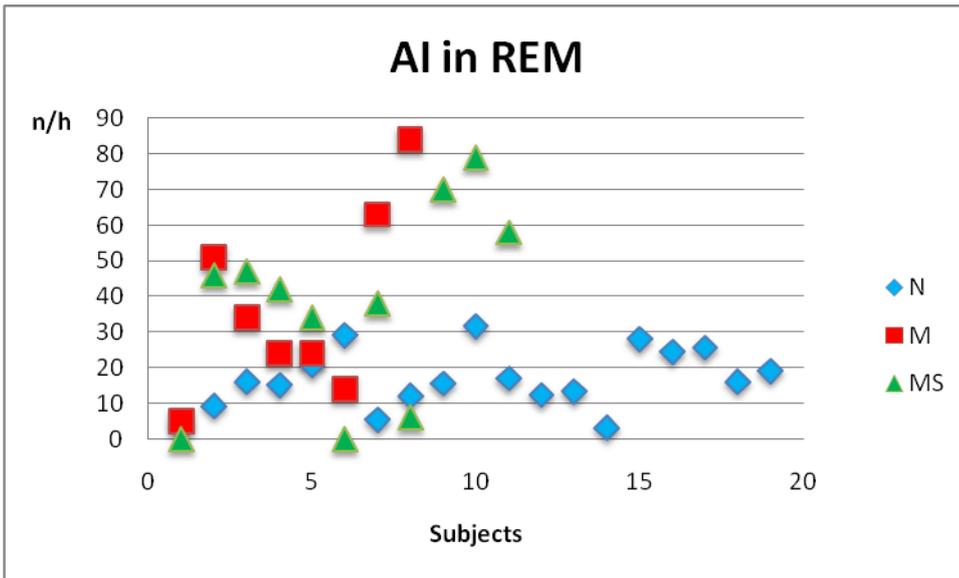


Figure A-12: Index of arousal (AI) in REM sleep in OSAS patients and normal subjects
 Abbreviations: N: normal subjects, M: mild OSAS patients, MS: moderate-severe OSAS patients

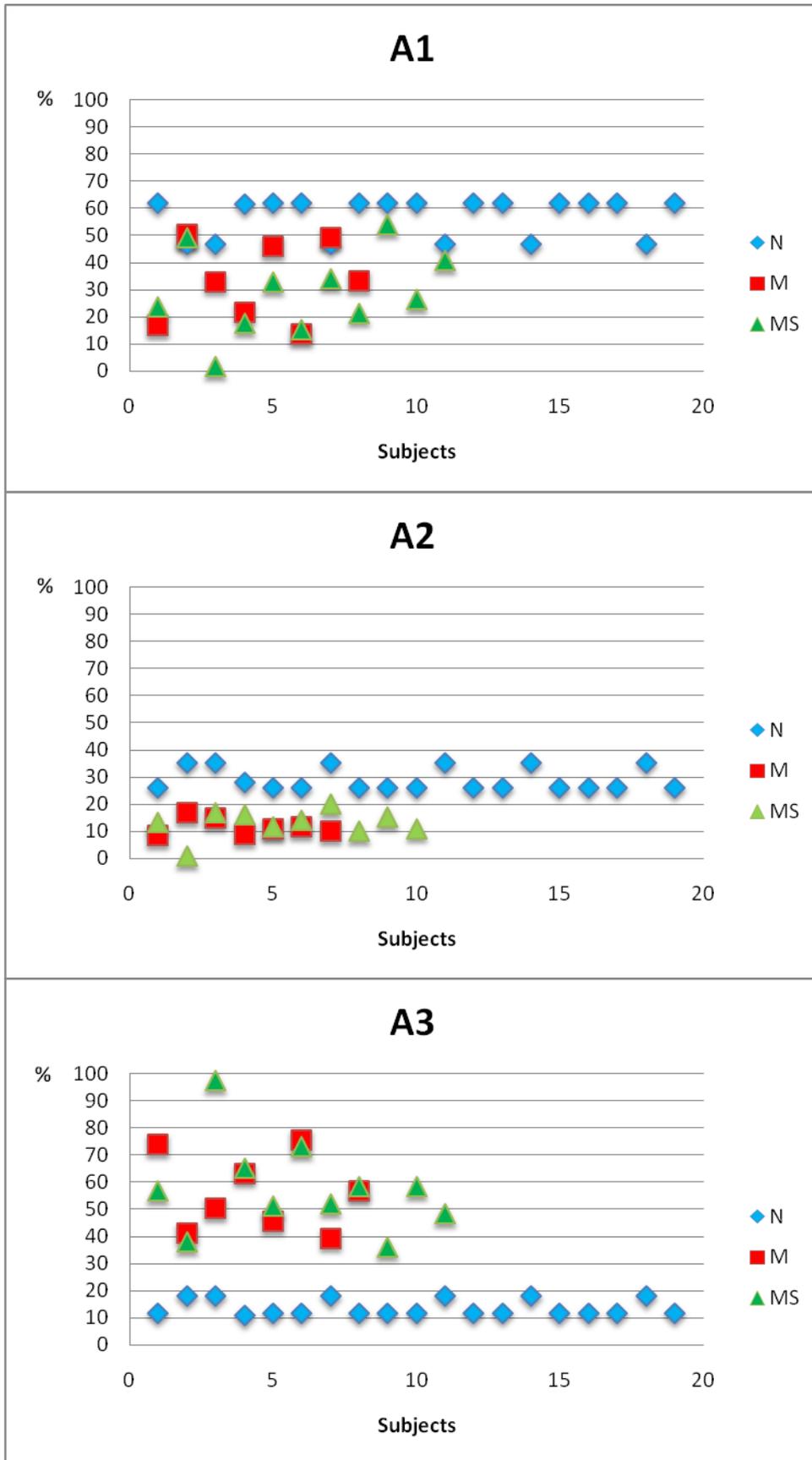


Figure A-13: Distribution of three subtype of CAP A phases in NREM sleep in OSAS patients and normal subjects

Abbreviations: N:normal subjects, M: mild OSAS patients, MS:moderate-severe OSAS patients

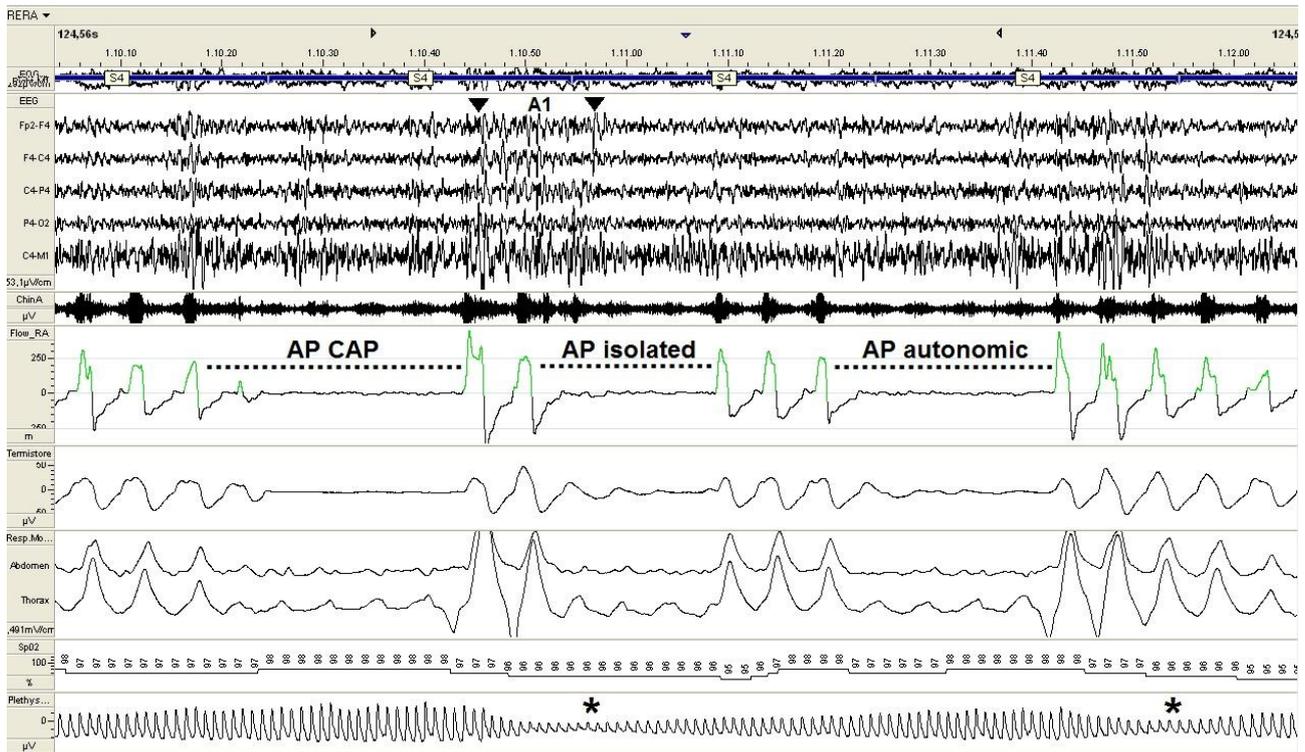


Figure A-14: Cerebral and autonomic responses to apnea events

Examples of apneas (dotted lines) in which airway re-opening occur 1) with CAP A phases (AP CAP), 2) without autonomic and cerebral response (AP isolated), 3) only with PWA drop (AP autonomic). CAP A phase is delimited by black triangles and PWA drops by asterisk.

Abbreviations. EOG: electrooculographic channels, EEG: electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer, Termistore: oronasal thermal sensor, Thorax: toracic effort by respiratory inductive plethysmogram, Abdomen: abdominal effort by respiratory inductive plethysmogram, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

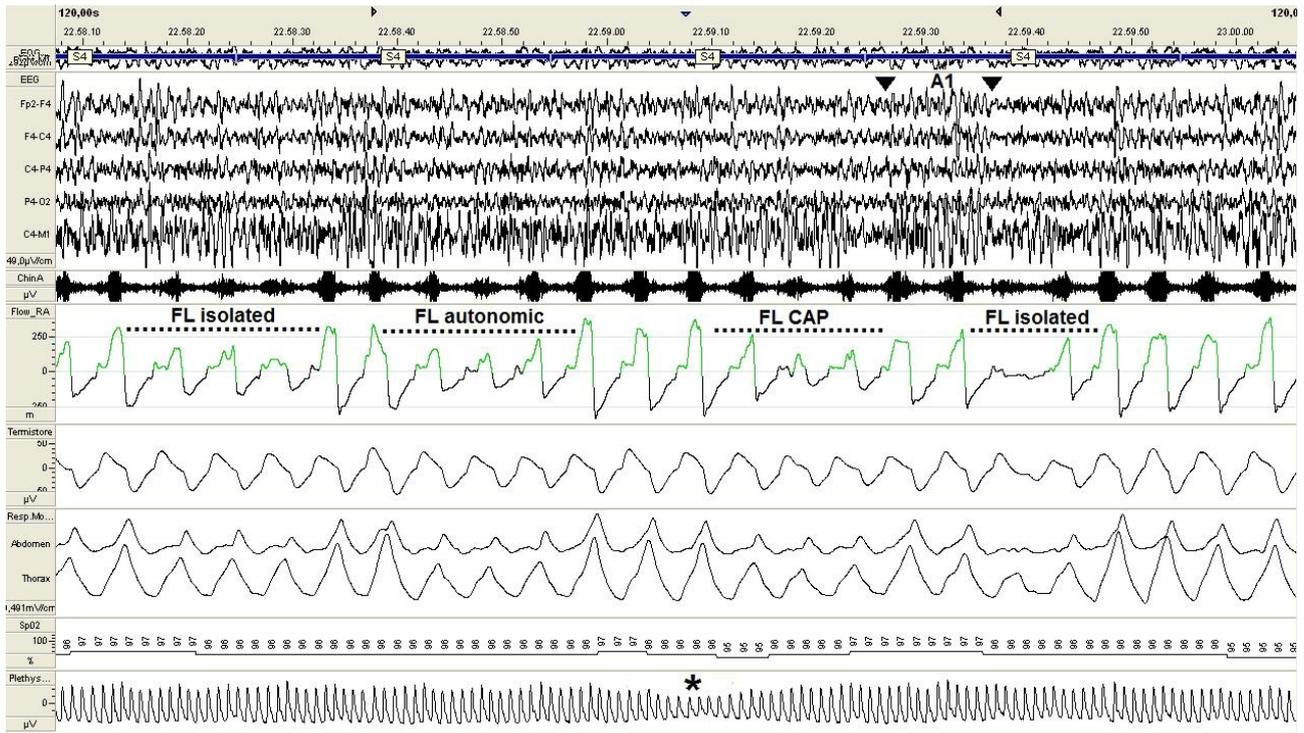


Figure A-16: Cerebral and autonomic responses to flow limitation events

Examples of flow limitation events (dotted lines) in which airway re-opening occur 1) without autonomic and cerebral response (FL isolated), 2) only with PWA drop (FL autonomic), 3). with CAP A phases (FL CAP) CAP A phases are delimited by black triangles and PWA drops by asterisk.

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer , Termistore: oronasal thermal sensor,Thorax: toracic effort by respiratory inductive plethysmogram, Abdomen: abdominal effort by respiratory inductive plethysmogram, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

Appendix: Section II

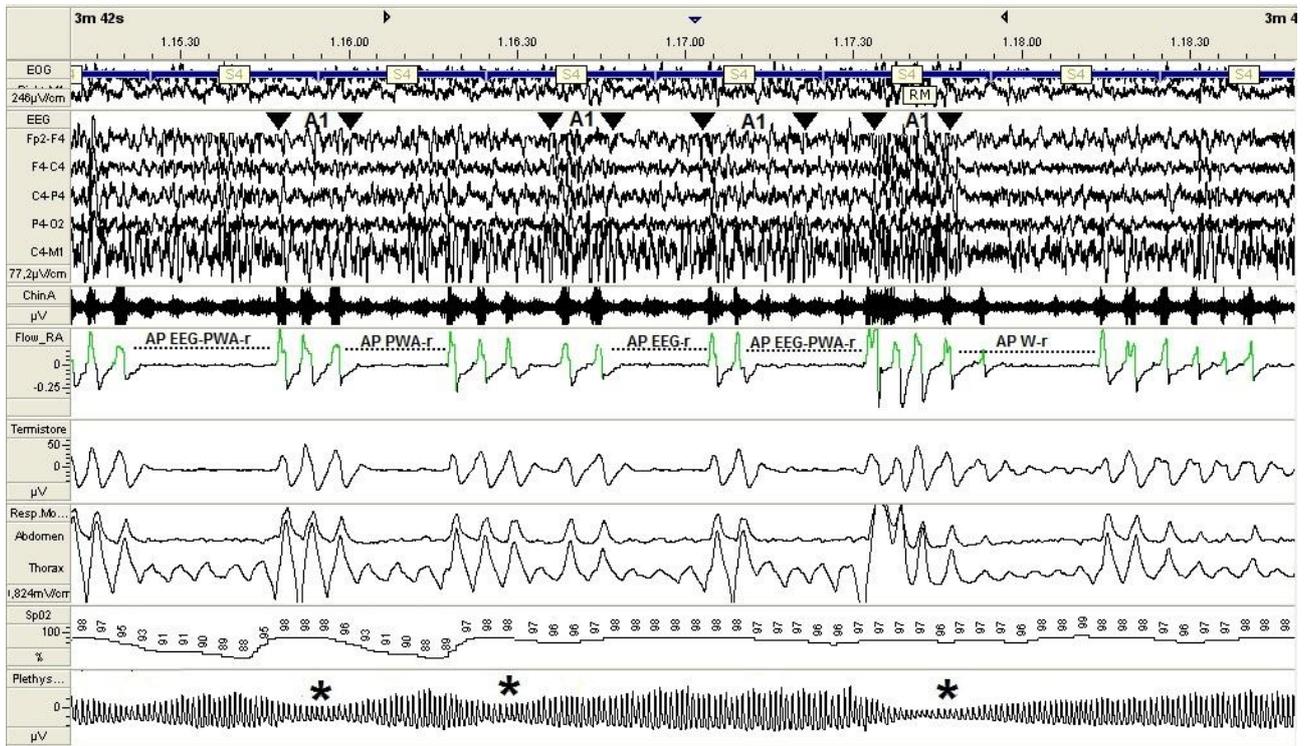


Figure A-17: Cerebral and autonomic responses to apnea events

Examples of apneas (dotted lines) in which airway re-opening occur 1) with dual response (AP EEG-PWA-r), 2) only with PWA drop (AP PWA-r), 3) only with CAP response (AP EEG-r) and 4) without autonomic and cerebral response (AP W-r). CAP A phase is delimited by black triangles and PWA drops by asterisk.

Abbreviations. EOG: electrooculographic channels, EEG: electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer, Termistore: oronasal thermal sensor, Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO₂: oxygen saturation, Plethys: finger photoplethysmogram.

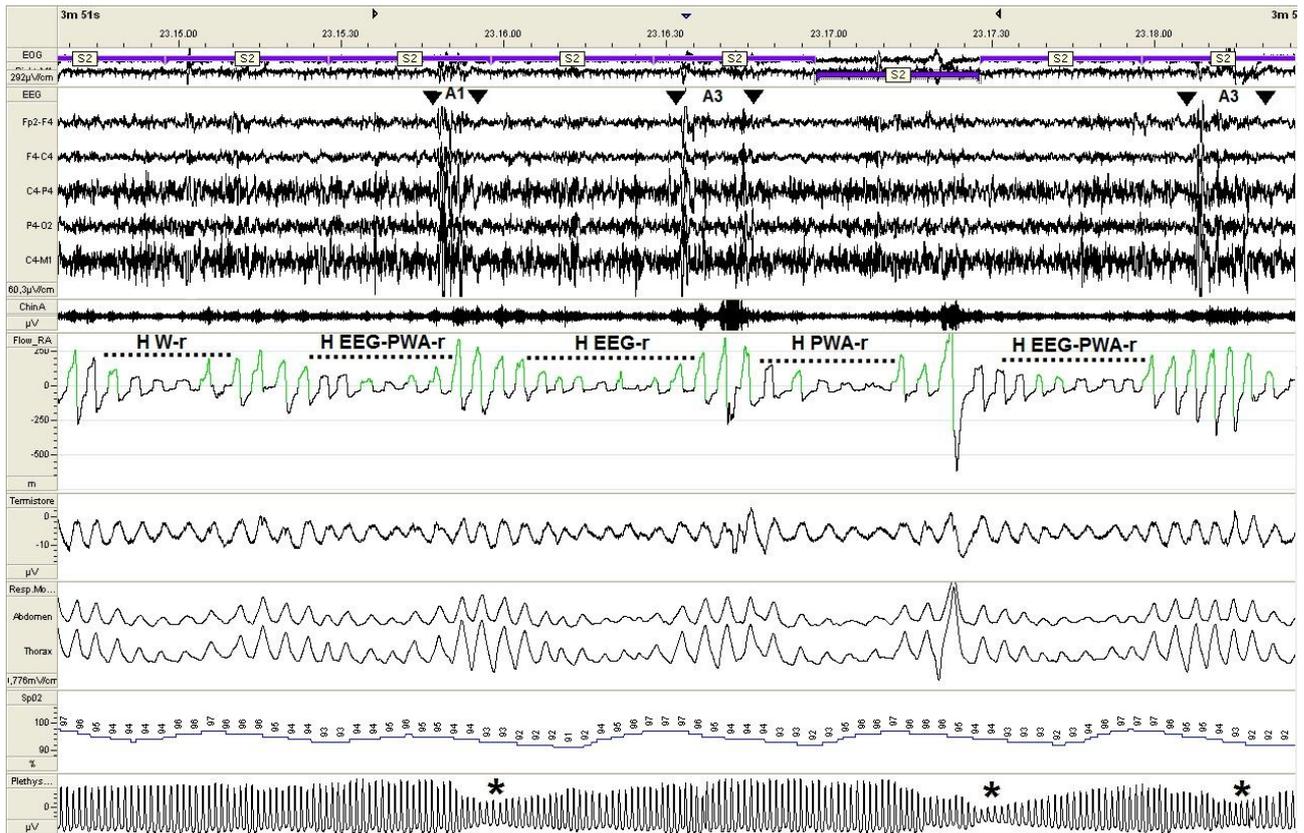


Figure A-18: Cerebral and autonomic responses to hypopnea events

Examples of hypopneas (dotted lines) in which airway re-opening occur 1) without autonomic and cerebral response (H W-r) 2) with dual response (H EEG-PWA-r), 3) only with CAP response (H EEG-r) and 4) only with PWA drop (H PWA-r). CAP A phase is delimited by black triangles and PWA drops by asterisk.

Abbreviations. EOG: electrooculographic channels, EEG: electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer, Termistore: oronasal thermal sensor, Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

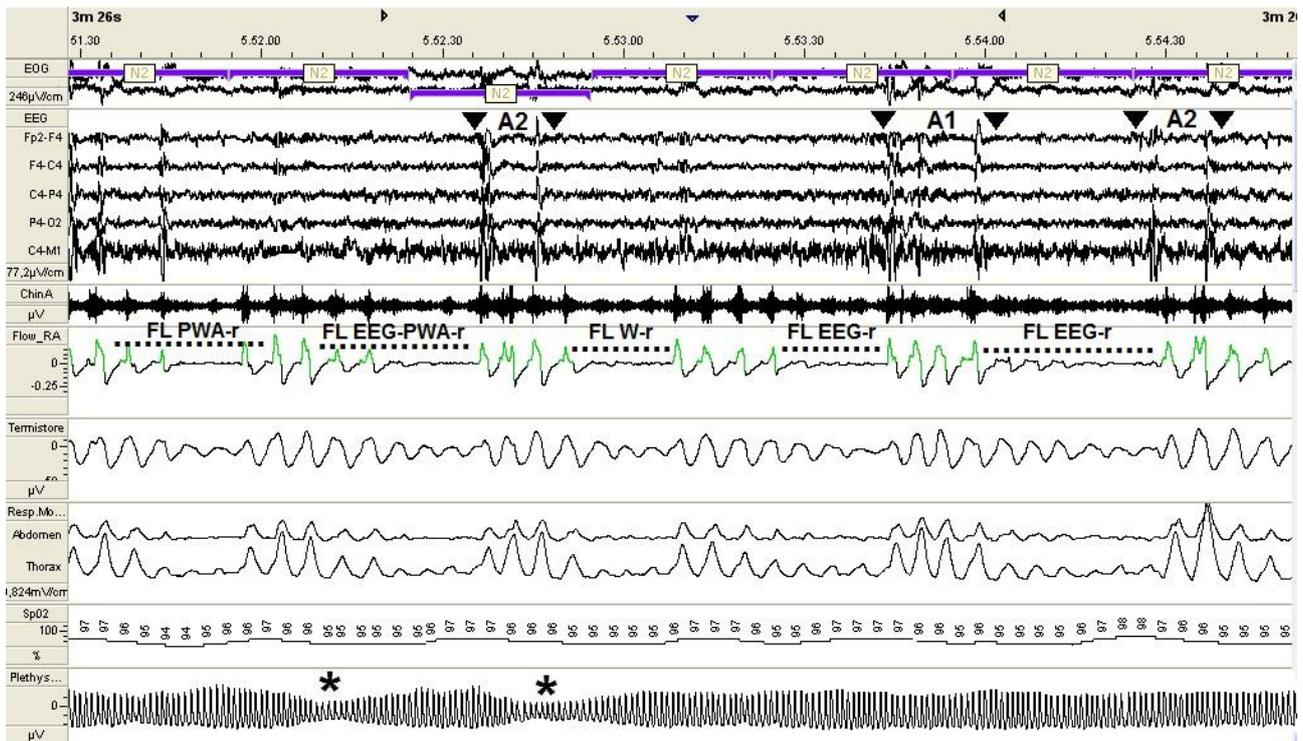


Figure A-19: Cerebral and autonomic responses to flow limitation events

Examples of flow limitation events (dotted lines) in which airway re-opening occur 1) only with autonomic response (FL PWA-r) 2) with dual response (FL EEG-PWA-r), 3) without cerebral and autonomic response (FL W-r) and 4) only with CAP response (FL EEG-r). CAP A phase is delimited by black triangles and PWA drops by asterisk.

Abbreviations. EOG: electrooculographic channels, EEG:electroencephalographic traces, Chin A: submental electromyogram, Flow RA: nasal air pressure transducer, Termistore: oronasal thermal sensor, Thorax: toracic effort by respiratory inductive plethysmograph, Abdomen: abdominal effort by respiratory inductive plethysmograph, SpO2: oxygen saturation, Plethys: finger photoplethysmogram.

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