Sleep

Evaluation of autonomic nervous system in sleep apnea patients using pupillometry under occlusal stress: a pilot study

Annalisa Monaco¹, Ruggero Cattaneo¹, Luca Mesin², Edoardo Fiorucci³, Davide Pietropaoli¹,⁴

¹Department of Life, Health and Environmental Sciences, Dental Unit, University of L’Aquila, Italy, ²Department of Electronics and Telecommunications, Politecnico di Torino, Italy, ³Dipartimento di Ingegneria Industriale e dell'Informazione e di Economia, University of L’Aquila, Italy, ⁴Department of Medicine, Case Western Reserve University, Cleveland, OH, USA

Aims: Recently, it has been proposed that obstructive sleep apnea syndrome (OSAS) is characterized by an imbalance in autonomic nervous tone. Pupil size has been considered a valid test for studying the autonomic nervous system (ANS). Pupillometry is a simple and non-invasive tool to assess the size and dynamics of the pupil. The purpose of this study was to evaluate, by pupillometry, the hypothesis that subjects with OSAS present ANS dysregulation.

Methods: The study group included 10 males aged between 40 and 50 years with polysomnographic diagnoses of mild OSAS. The control group included 10 males with similar ages with an apnea–hypopnea index (AHI) of less than 5, after polysomnography. Pupillometry was performed by digital infrared pupillometer (25 frame/s). Recordings were processed to measure the area of the pupil frame by frame. The subjects underwent four subsequent recordings: infrared light at rest mandible position (RP); infrared light at forced habitual occlusion (FHO); yellow-green light at RP; and yellow-green light at FHO. According to literature, linear and non-linear information was extracted from the recordings.

Results: As expected, the two groups did not differ statistically in age and body mass index (BMI), while there was a significant difference in the AHI. In the within-group comparison of pupil size, there were significant differences between RP and FHO under infrared conditions in the control group. There was a significant difference in the determinism percentage (Det%) in the RP infrared condition between the control and OSAS groups.

Conclusions: The results of the current study confirm ANS dysregulation in OSAS patients and provide a new possible strategy for studying this pathology by using pupillometry through linear and non-linear mathematical models.

Keywords: Autonomic nervous system, ANS, Obstructive sleep apnea syndrome, OSAS, Pupillometry, ANS dysregulation, Forced habitual occlusion, Rest mandible position

Introduction

Recently, it has been proposed that patients with obstructive sleep apnea syndrome (OSAS) are characterized by an imbalance in autonomic nervous tone. In particular, some authors suggested that sympathetic hypertonus is positively correlated with daytime sleepiness.¹ Sympathetic hypertonus could correspond to impaired reactions to several physiological stimuli, which are dependent on the severity of OSAS.² On the other hand, sympathetic hyperactivity would largely be responsible for the heart and metabolic diseases that frequently affect these patients.³ Some results⁴ suggested that autonomic abnormalities suggestive of decreased adrenergic tone are associated with mild obstructive sleep apnea (OSA) and may potentially be secondary to OSA, but may also precede development of OSA.

Parasympathetic system dysfunction may play a key role in the dysregulation of the autonomic nervous system (ANS) in OSAS patients.⁵ Indeed,
heart rate acceleration during non-rapid eye movement sleep is related to an increase in sympathetic tone, but parasympathetic tone accommodation fails in OSAS patients. OSAS patients have a continuous resetting of their sympathetic tone. Even when the airway reopens, they have less leeway to decrease their parasympathetic tone, which is due to the need for continuously balancing the sympathetic tone resetting. The lifting of the parasympathetic modulation at the end of resistive breathing is an important component of changes in heart rate, and it plays a more important role than sympathetic stimulation. Resistive breathing without hypoxemia during wakefulness causes a fall in arterial pressure, associated with a decline in muscle sympathetic nerve activity, allowing the parasympathetic tone to be the main regulator.6,7

The dynamics of the pupil shows apparently random movements and changes in size, even in the presence of a constant stimulation. Recently, the study of pupil size has been considered a valid test for studying the ANS.8 Pupil size is controlled by complex interaction between the sympathetic and parasympathetic branches of the ANS: the first uses mainly adrenergic pathways, and the second uses cholinergic pathways. Both muscles of the iris (i.e. the sphincter and dilator) receive reciprocal innervations from the two branches of the ANS, allowing for contraction and inhibition (or relaxation). Parasympathetic cholinergic fibers, coming from the Edinger–Westphal nucleus, supply the iris sphincter and act in the contraction of the muscle and the consequent reduction of pupil size. At the same time, the sphincter receives beta-adrenergic innervations that are capable of reducing the contraction by inducing relaxation of the muscle.9,10 In humans, pupil dilation obtained by beta-adrenergic inhibition of the sphincter can equal one-third of the maximum physiological dilation.11 On the other hand, the iris dilator muscle receives a predominant adrenergic sympathetic motor innervation (Budge’s Cilio Spinal Center) causing contraction and the consequent increase in pupil size. Dilator muscle contraction is mediated by alpha-adrenergic receptors, and inhibition or relaxation may be exercised by muscarinic receptors, and, although not yet fully documented, by beta-adrenergic innervations.12–16

Therefore, central modulation of sympathetic and parasympathetic activity results in a dynamic equilibrium of pupillary size. Increases in sympathetic activity are characteristically accompanied by central inhibition of parasympathetic activity. A strong decrease in central nervous system activation was observed in sleep-deprived normal subjects.17

Pupillometry is a simple and non-invasive tool to assess the size and dynamics of the pupil. It is considered a reliable tool in the study of drug effects.18–20 Pupil size is significantly correlated with heart rate variability,21 indicating its usefulness for assessing ANS dysregulation under clinical conditions in which the ANS is involved.22–27 Some oscillation frequencies of different systems affected by ANS control (e.g. cardiovascular and respiratory systems) are coupled28,29 and the pupil shares some of the common rhythms.30–33 Moreover, pupillary indexes in healthy normal subjects were found to be positively correlated with the level of daytime alertness and were similar to daytime variations in the multiple sleep latency test.34 Pupillography can be used to document the therapeutic effect of positive airway pressure in sleep apnea patients.35

The purpose of this study was to evaluate by pupillometry, the hypothesis that subjects with OSAS present autonomic imbalance, and to determine which of the two branches of the ANS is more involved. In this respect, mean pupil size and non-linear parameters, extracted using recurrence quantification analysis (RQA), were used to characterize pupil oscillation dynamics in stationary light conditions. RQA is a specific non-linear technique, which was introduced to study the non-linear dynamics of various natural and artificial systems including biological signals.36

This technique was used to study ANS activity beyond that in OSAS,37 but also in other systemic conditions like diabetes38 and temporomandibular disorders.36 Moreover, as already reported in previous works,36,39 muscle activation (forced habitual dental occlusion) was also considered, since it is able to weakly activate the sympathetic component of the autonomic system.

Materials and Methods

Subjects

This study was conducted in accordance with the Declaration of Helsinki. The Committee on Ethics in Science of the University of L’Aquila, L’Aquila, Italy, approved the study. Informed consent was obtained from each subject.

The study group included 10 males aged between 40 and 50 years [mean: 43.62 years, standard deviation (sd): 4.64 years] with polysomnographic diagnoses of mild OSAS [mean apnea–hypopnea index (AHI) 13.63, sd: 4.12], and a mean body mass index (BMI) of 27.52 (sd 3.13). The study group was compared with 10 male subjects in the control group with similar ages (mean age: 41.78, sd 7.45) and BMIs
The subjects in the control group were accepted only after polysomnography showed that they had an AHI of less than 5 (mean: 2.14, sd: 0.18). Both groups had similar exercise activity (3 hours/week).

Subjects were excluded from the study if they met one or more of the following criteria: systemic or metabolic diseases; eye diseases or visual defects; history of local or general trauma; neurological or psychiatric disorders; muscular diseases; cervical pain; bruxism, diagnosed by the presence of parafunctional facets and/or anamnesis of parafunctional tooth clenching and/or grinding; pregnancy; use of beta-blocking, anticholinergic, anti-inflammatory, analgesic, anti-depressant, opioid, myorelaxant, and other drugs that could affect ANS; smoking; alcohol misuse; fixed or removable prosthesis; and fixed restorations that affected the occlusal surfaces.

Before the experiment were carefully controlled illumination levels, pharmacological assumption (no drugs, no caffeine, no smoking 6 hours ago), and physiological state.

**Pupillometry**

Pupillometry was performed at the same time of the day with a table-mounted infrared pupillometer (Oculus System; Inventis SRL, Padova, Italy), which was composed of two infrared charge-coupled device cameras (resolution of 720 x 576 pixels, 256 gray levels) mounted on a light helmet (1.5 kg), with a sampling frequency of 25 frame/s. To stabilize accommodation, the subjects were asked to focus their eyes on the light point in the pupillometer.19

Assessment of pupil size was performed under light conditions by illuminating the eyes with a yellow-green LED with a wavelength of 740 nm, and under dark conditions that were obtained with an infrared diode with a wavelength of 880 nm. Pupillometric recordings were acquired in digital form and processed to measure the area of the pupil frame by frame, and the area was expressed as a number of pixels. A template was positioned on the computer screen, enabling corrections to be made for eye positioning to avoid errors due to different positions of the pupils. Pupillometry was performed with the subjects in the horizontal supine position on a bed for 3 minutes, to adapt to the temperature and humidity of the room, as well to reduce anxiety. Then the pupillometer was applied and maintained until the end of the recording session. Recording sessions started at 13:00 and ended at 15:00, during the period in which the dynamics of the pupil may indicate a greater degree of daytime sleepiness.40

**Recording procedure**

The subjects underwent four subsequent recordings, each 30 seconds in length:

1. infrared light at rest mandible position (RP);
2. infrared light at forced habitual occlusion (FHO);
3. yellow-green light at RP;
4. yellow-green light at FHO.

The sequence of tests was assigned randomly. At the end of each test, a period of 1 minute followed, during which the subject was asked to keep their eyes closed. Each new recording started 15 seconds after the subjects opened their eyes.

FHO was standardized by surface electromyography (SEMG). Disposable electrodes (Duotrode, bipolar surface electrodes Ag–AgCl, 20 mm center-to-center distance; Myotronics-Noromed, Inc., Tukwila WA, USA) were used for the SEMG recordings on the right and left masseters. The electrodes were connected to SEMG equipment (K7/EMG; Myotronics-Noromed, Inc.). For each subject, a pretest established the average value of SEMG amplitude corresponding to maximum voluntary clenching. During the FHO tests, the SEMG values were maintained with verbal instructions from the operator between 30 and 50% of the maximum voluntary clenching.

**Signal processing**

The frames recorded by the cameras were processed as described in a previous work.39 In summary, the pupil was identified by the region growing algorithm, and its size was computed as the sum of pixels belonging to it. The pupil area was considered as a time series extracted from a deterministic physiological system. Time series embedding was performed according to previously described methods,41 and the time series was converted into a vector of delayed coordinates (phases), with the embedding dimension equal to 6 and delay equal to 10, as described by Mesin.39 Non-linear information to be included in pupil dynamics was suggested previously.39

In particular, indexes from RQA were used to characterize data from healthy subjects.39 RQA provides quantitative indexes related to the number and duration of recurrences of the trajectory of a dynamical system in the phase space.42 Such recurrences are events in which the system revisits a point.
close to another where it passed before. They are identified in the recurrence plot, which is a binary map indicating the reference time and the delay of neighboring points. To exclude neighboring points that are close in time, a minimum time interval of 10 seconds between different points (called the Theiler window) was considered in the recurrence plot.

Different indexes can be extracted from the recurrence map: the recurrence rate (RR) and the percentage of determinism (DET, percentage of recurrence points forming diagonal lines in the recurrence plot of minimal length \( L_{\text{min}} \)). The RR and DET are defined as:

\[
RR = \frac{1}{N^2} \sum_{i,j=1}^{N} R(i,j), \quad DET = \frac{\sum_{i=1}^{L_{\text{min}}} IP(j) \sum_{i,j=1}^{N} R(i,j)}{N^2}
\]

where \( N \) is the number of entries of the recurrence map \( R \) and \( P(l) \) is the frequency distribution of the lengths of the diagonal lines. The recurrence rate provides information on the number of recurrences and on the degree of self-similarity of the trajectory; the determinism is related to the predictability of the system.

Here, instead of considering two different indexes, their information was included in DET, by computing it after choosing the threshold under which points are considered neighbors in order to fix the value of RR to 0.5. Moreover, the value of DET in equation (1) depends on the parameter \( L_{\text{min}} \), which measures the length of the diagonal lines, i.e. the time duration of a recurrent trajectory. The DET index decreases monotonically; as such, a time duration is increased. For the investigated time series, the value of DET saturated to a constant for values of \( L_{\text{min}} \) lower than 20 was considered. A robust estimation of the index was computed as the average of the values obtained for \( L_{\text{min}} \), varying in the range 2–20.

**Statistical analysis**

Statistical analysis was performed using STATA 10 (StataCorp LP, College Station, TX, USA) on average pupil size and on percentage of determinism, which were computed for 30 seconds of recording. The ratio between pupil size and percentage of determinism in FHO and RP (referred to as the FHO/RP ratio in the following) and yellow-green and infrared (light/darkness ratio) lighting were calculated. The Shapiro–Wilk test revealed a normal distribution of data. Within-group differences in pupil size and the ratios were analyzed using paired \( t \)-tests, while differences in pupil size between groups were tested using an unpaired \( t \)-test. The level of significance was set at \( P<0.05 \) for all tests. Results are expressed as the mean and sd.

**Results**

As expected, the two groups did not differ statistically in age and BMI, while there was a significant difference in the AHI (Table 1). Statistical comparisons between groups showed no significant differences in the absolute values for pupil dimension (mean size) in RP and FHO, in either yellow-green or in infrared lighting. In addition, there were no significant differences within groups comparing RP and FHO in yellow-green light.

In the within-group comparison of pupil size, there were significant differences between RP and FHO under infrared conditions in the control group. In particular, in the control group, the pupils had a significantly larger size (\( P=0.016 \) in FHO compared to RP. In the OSAS group, the size of the pupil was smaller in FHO compared to RP (5183.19 versus 5353.25), but this difference was not significant (Tables 2 and 3).

There was a significant difference in the determinism percentage (Det%) in the RP infrared condition between the control and OSAS groups (\( P=0.007 \)). No other statistical differences in Det% were observed in the between and within group comparisons (Table 4).

The pupil size FHO/RP ratio did not present significant differences under yellow-green light conditions in the between groups comparison. In contrast, under the infrared light condition, the comparison between groups showed a significant difference (\( P=0.037 \)) with a larger ratio in the control group (1.045) compared to the OSAS group (0.931) (Table 5).

There was no significant difference in the Det% FHO/RP ratio between groups under yellow-green light conditions. Under infrared conditions, the Det% FHO/RP ratio was significantly lower (\( P=0.046 \)) in the control group (Table 6).

The pupil size light/darkness ratio did not show any significant difference in the between groups comparison (Table 7). The Det% light/darkness ratio

| Table 1 Clinical and epidemiological characteristics of the control and OSAS groups |
|---------------------------------|---|---|---|
|                                | AHI | BMI | Age (years) |
| Control group                  | 2.07 (1.58) | 26.24 (1.57) | 60.01 (14.31) |
| OSAS group                     | 12.84 (2.11) | 27.54 (2.67) | 56.43 (8.55) |
| \( t \)-test                   | 0.000001 | 0.11 | 0.24 |

Note: AHI=apnea-hypopnea index; BMI=body mass index.
showed a significant difference ($P=0.30$) in RP in the between group comparison, presenting a lower value in the control group (1.017) compared to the OSAS group (1.083) (Table 8).

**Discussion**

The present study provides preliminary pupillometric data confirming ANS dysregulation in OSAS patients. In particular, it should be noted that: (1) OSAS patients show pupil size values comparable to controls in RP infrared and in RP light conditions; (2) OSAS patients show Det% values lower than controls in the RP infrared condition; (3) under the FHO infrared stress condition, the pupil size of OSAS subjects behaves differently compared to the control subjects. In the control group, the pupil size increases, whereas in OSAS patients, the pupil size decreases at FHO in infrared lighting.

In general, the size and determinism of the OSAS group does not behave in a different way than in the controls under the yellow-green light condition. On the other hand, in darkness, the two groups behave differently. There is a lesser degree of determinism in pupil oscillation dynamics and a lower reaction to light stimuli (FHO) activating ANS in the OSAS group.

As a result of the balance between the two branches of the ANS in the control of pupil size and dynamics, the presence of a relative hypertonus of the sympathetic component could result in an increase in pupil size in stationary conditions of darkness. The resting size of the pupil diameter in darkness and in response to emotional changes is determined primarily by changes in sympathetic activity, and a reduction in diameter is a sign of diminished sympathetic outflow to the iris muscles. Sympathetic tone has been linked to variation in pupil size under physiological conditions and in clinical pharmacology studies in healthy subjects. A reduction in sympathetic tone has been correlated with a reduction in pupil size in physiological aging, congenital dysautonomia, and in diseases hypothesized to have deficient activity of the sympathetic system.

Some authors have suggested that mild OSAS patients suffer from autonomic abnormalities suggestive of autonomic dysfunction. In the current study, sympathetic hypertonus in the OSAS subjects could occur with an increase in mean pupil size in darkness or as a relatively smaller reduction in size to light (light/darkness ratio). The first case is due to direct action on the adrenergic dilator muscle

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**Table 2** Pupil sizes and percentages of determinism values under different conditions in the control and OSAS groups

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control Group</th>
<th>OSAS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP yellow-green light</td>
<td>2497.15 (978.72)</td>
<td>2754.97 (1425.37)</td>
</tr>
<tr>
<td>FHO yellow-green light</td>
<td>5431.32 (1391.83)</td>
<td>5732.47 (1840.53)</td>
</tr>
<tr>
<td>RP infrared light</td>
<td>2264.51 (1741.06)</td>
<td>2364.51 (1923.83)</td>
</tr>
<tr>
<td>FHO infrared light</td>
<td>5353.25 (1394.34)</td>
<td>5183.19 (1923.83)</td>
</tr>
</tbody>
</table>

Note: Mean Size = mean pupil size, Det% = percentage of determinism.
of the pupil, and the second case is due to an increase in beta-adrenergic inhibition on the sphincter muscle.9,10 The authors did not observe a different dimension of the pupil under rest in the dark and the light between the control group and the OSAS group. According to these data, it is not possible to suggest that subjects with mild OSAS present daytime sympathetic hypertonus influencing pupil size in basic conditions. On the other hand, functional mental53,54 and physical55,56 loads involve activation of the sympathetic system, which results in an increase in pupil size in the dark. The increase in the pupil size in the darkness, determined by emotional and muscle demands, is due mostly to the simultaneous inhibition of cholinergic (parasympathetic) pathways by the adrenergic system (sympathetic).57 Clenching produces sympathetic activation,39 partly via an increase of blood flow and pressure of the head58,59 and partly through direct sympathetic innervations of neuromuscular spindles of the masticatory muscles.60–64 Parasympathetic inhibition defect involves failure to increase pupil size in darkness during the forced habitual occlusion (FHO).36

The authors’ data on OSAS subjects seem to confirm these observations. In fact, the statistical comparison within the control group showed a significant increase in pupil size during FHO in darkness and a FHO/RP greater than 1. Meanwhile, the OSAS group showed a non-significant reduction in pupil size and a FHO/RP less than 1. The comparison between groups of the FHO/RP ratio showed a statistically significant difference.

Data that refer to the Det% can be interpreted in a similar way. Many biological signals are characterized in stationary conditions by fluctuations over time of their absolute values, which often have non-linear characteristics. The complex dynamics of biological signals, such as those derived from pupil dynamics suggest the necessity of studying this system using non-linear analysis techniques.65 The fluctuations have been studied by parameters derived from RQA, as the Det%. RQA was introduced to study the non-linear dynamics of various natural and artificial systems, including biological signals.66–68 One of the advantages of this technique is the ability to analyze a relatively short time series of non-linear

### Table 3 Pupil sizes (pixels) and statistical comparisons under different conditions in the two groups

<table>
<thead>
<tr>
<th>Mean size</th>
<th>RP in yellow-green light condition</th>
<th>FHO in yellow-green light condition</th>
<th>RP in infrared light condition</th>
<th>FHO in infrared light condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>2497.15 (978.72)</td>
<td>2754.97 (1425.37)</td>
<td>5431.32 (1591.83)</td>
<td>5732.47* (1840.53)</td>
</tr>
<tr>
<td>OSAS group</td>
<td>2364.51 (1741.06)</td>
<td>2243.47 (1555.85)</td>
<td>5353.25 (1304.34)</td>
<td>5183.19 (1923.83)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.30</td>
<td>0.23</td>
<td>0.46</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Note: Standard deviations are shown in brackets. *Comparison between RP and FHO in infrared light condition within the control group: P=0.016.

### Table 4 Determinism percentages and statistical comparisons under different conditions in the two groups

<table>
<thead>
<tr>
<th>Det%</th>
<th>RP in yellow-green light condition</th>
<th>FHO in yellow-green light condition</th>
<th>RP in infrared light condition</th>
<th>FHO in infrared light condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.953 (0.032)</td>
<td>0.937 (0.058)</td>
<td>0.937 (0.036)</td>
<td>0.911 (0.060)</td>
</tr>
<tr>
<td>OSAS group</td>
<td>0.927 (0.040)</td>
<td>0.923 (0.027)</td>
<td>0.864 (0.048)</td>
<td>0.894 (0.069)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.06</td>
<td>0.25</td>
<td>0.007</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Note: Det% = percentage of determinism. Standard deviations are shown in brackets. Significance level at P=0.05.

### Table 5 FHO and RP ratios of pupil size in the control and OSAS groups

<table>
<thead>
<tr>
<th>Mean size</th>
<th>Yellow-green light</th>
<th>Infrared light</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHO/RP ratio in control group</td>
<td>0.996 (0.149)</td>
<td>1.045 (0.020)</td>
</tr>
<tr>
<td>FHO/RP ratio in OSAS group</td>
<td>1.002 (0.169)</td>
<td>0.931 (0.056)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.46</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Note: Mean size = mean pupil size. Statistical comparison between the two groups. Significance level at P=0.05. Standard deviations are shown in brackets.

### Table 6 FHO and RP ratios of determinism percentage in the control and OSAS groups

<table>
<thead>
<tr>
<th>Det%</th>
<th>Yellow-green light</th>
<th>Infrared light</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHO/RP ratio control group</td>
<td>0.982 (0.018)</td>
<td>0.978 (0.022)</td>
</tr>
<tr>
<td>FHO/RP ratio OSAS group</td>
<td>0.994 (0.008)</td>
<td>1.034 (0.019)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.29</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Note: Det% = percentage of determinism. Statistical comparison between the two groups. Significance level at P=0.05. Standard deviations are shown in brackets.
The data of the current study agree with the above observation. The control group showed decreases in FHO Det% compared to RP Det% in the infrared condition. For this reason, the FHO/RP ratio of Det% was lower than 1. In the OSAS group, there were increases in FHO Det% compared to RP Det% in the infrared condition, and the FHO/RP ratio was higher than 1. The comparison between groups of the FHO/RP ratio shows significant differences. These data suggest that during physiologic activation (FHO), the control group’s Det% increases. Accordingly, the complexity of pupil dynamics decreases (FHO/RP lower than 1), and the OSAS group Det% decreases, showing an increase in pupil dynamics complexity.

The authors’ data cannot confirm the suggestion that in pathological conditions or aging, the dynamics of biological signals showed lower complexity and an increase in determinism. In fact, considering OSAS as a pathologic condition, the authors expected that the Det% in the dark RP would be higher in the OSAS group compared to the control group. Instead, data coming from RP in the infrared condition showed a significant decrease of OSAS Det% compared to the control group. The authors’ data suggest that the pupil non-linear parameter in OSAS patients shows a lower rate of complexity than control subjects. The RP Det% value in the infrared condition shapes the results of the FHO/RP ratio, light/darkness ratio, and the Det% comparison between the groups. On the other hand, the range of non-linear parameters, such as Det%, which includes many signals, is frequently unknown. In this sense, terms such as an increase or decrease in determinism are relative and may not have the same value in different experimental conditions. It is possible that this parameter has a more functional than pathogenetic significance when measuring the efforts of the OSAS subjects to maintain gaze in the dark without visual references. According to this interpretation, the reduction of determinism, and the corresponding increase in complexity, may be a sign of destabilization of dynamics towards chaotic or random behavior.

Taken together, the pupil size and determinism data show that the OSAS subjects show different behaviors compared to the control subjects under infrared conditions. These data indicate that OSAS subjects could suffer from a dysregulation of vegetative control probably due to difficulties in inhibiting cholinergic pathways during activation of adrenergic ones at the pupil level to adequately respond to activation (lack of light and muscular stress).

The results of the current study are limited if the changes observed are due to the primary defect of ANS or if they are due to the peripheral effects on nerve tissue or muscle or metabolic deficits. The selected sample, although small, did not have metabolic disorders, and the clinical and epidemiological characteristics were comparable with those of the control subjects. Subjects with low apnea index (mild OSAS) and without clinical disorders to exclude the effects of metabolic alteration on tissues were chosen for this study. The interpretation of the results of the current study needs more data because the sample is too small to draw definitive conclusions. Furthermore, this study used pupillometry to monitor the activity of ANS without comparing the pupillometric data to other data from signals previously used to study ANS in the literature (e.g. HRV and electrodermal activity). This limitation is one reason why the authors can only suggest a speculative interpretation of the data. The authors’ future work aims to confirm these results. For example, future work will compare OSAS patients suffering from different severity of OSAS, or compare patients treated with MAD or CPAP with untreated patients. Future investigations could help doctors understand if severity of the OSAS is related to ANS dysregulations. This work can be considered a pilot study. If these results are confirmed, this method can provide a useful, rapid, and non-invasive tool to test ANS behavior in OSAS patients and to quickly monitor the response to therapy.

### Table 7 Light/darkness ratios of pupil size at RP and FHO

<table>
<thead>
<tr>
<th>Mean size</th>
<th>RP</th>
<th>FHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light/darkness ratio control group</td>
<td>0.453 (0.036)</td>
<td>0.467 (0.041)</td>
</tr>
<tr>
<td>Light/darkness ratio OSAS group</td>
<td>0.405 (0.064)</td>
<td>0.435 (0.058)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.25</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Note: Mean size= mean pupil size. Comparison between groups. Significance level at P=0.05. Standard deviations are shown in brackets.

### Table 8 Light/darkness ratios of determinism percentage at RP and FHO

<table>
<thead>
<tr>
<th>Det%</th>
<th>RP</th>
<th>FHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light/darkness ratio control group</td>
<td>1.017 (0.017)</td>
<td>1.032 (0.028)</td>
</tr>
<tr>
<td>Light/darkness ratio OSAS group</td>
<td>1.083 (0.028)</td>
<td>1.049 (0.029)</td>
</tr>
<tr>
<td>t-test</td>
<td>0.030</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Note: Det%=percentage of determinism. Comparison between groups. Significance level at P=0.05. Standard deviations are shown in brackets.
Disclaimer statements

Contributors MA and CR conception and design of the study; CR and ML analysis and interpretation of data; EF and DP drafting the article; all authors approved the final version of the manuscript.

Funding None.

Conflicts of interest None.

Ethics approval Committee on Ethics in Science of the University of L’Aquila.

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