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## **Impaired post-sleep apnea autonomic arousals in patients with drug-resistant epilepsy**

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### **Abstract**

**Objective:** Sudden and unexpected deaths in epilepsy (SUDEP) pathophysiology may involve an interaction between respiratory dysfunction and sleep/wake state regulation. We investigated whether patients with epilepsy exhibit impaired sleep apnea-related arousals.

**Methods:** Patients with drug-resistant (N=20) or drug-sensitive (N=20) epilepsy and obstructive sleep apnea, as well as patients with sleep apnea but without epilepsy (controls, N=20) were included. We explored (1) the respiratory arousal threshold based on nadir oxygen saturation, apnea-hypopnea index, and fraction of hypopnea among respiratory events; (2) the cardiac autonomic response to apnea/hypopnea quantified as percentages of changes from the baseline in RR intervals (RRI), high (HF) and low (LF) frequency powers, and LF/HF.

**Results:** The respiratory arousal threshold did not differ between groups. At arousal onset, RRI decreased (-9.42%) and LF power (179%) and LF/HF ratio (190%) increased. This was followed by an increase in HF power (118%),  $p < 0.05$ . The RRI decrease was lower in drug-resistant (-7.40%) than in drug-sensitive patients (-9.94%) and controls (-10.91%),  $p < 0.05$ . LF and HF power increases were higher in drug-resistant (188%/126%) than in drug-sensitive patients (172%/126%) and controls (177%/115%),  $p < 0.05$ .

**Conclusions:** Cardiac reactivity following sleep apnea is impaired in drug-resistant epilepsy.

**Significance:** This autonomic dysfunction might contribute to SUDEP pathophysiology.

### Highlights

1. The respiratory arousal threshold does not differ between apneic patients with and without epilepsy
2. The arousal related heart rate increase after obstructive apnea is less pronounced in patients with drug-resistant epilepsy
3. Late post-arousal parasympathetic activation is increased in patients with drug-resistant epilepsy

### Key words

Sleep apnea, Epilepsy, SUDEP, Arousal, Heart rate

### Abbreviations

American Academy of Sleep Medicine: AASM

Apnea index: AI

Apnea-hypopnea index: AHI

Arousal index: ArI

Beck Depressive Inventory: BDI

Body Mass Index: BMI

Continuous positive air pressure: CPAP

Drug-resistant epilepsy: DRE  
Drug-sensitive epilepsy: DSE  
Epworth Sleepiness Scale: ESS  
Heart rate variability: HRV  
Hypopnea index: HI  
Non-rapid-eye-movement sleep: NREM sleep  
Non-rapid-eye-movement sleep stage 2 : N2  
Non-rapid-eye-movement sleep stage 3 : N3  
Obstructive Sleep Apnea: OSA  
Oxygen desaturation index: ODI  
Polysomnography: PSG  
Rapid-eye-movement sleep: REM sleep  
RR interval: RRI  
Standard Deviation: SD  
Sleep onset latency: SOL  
Sudden Unexpected Death in Epilepsy: SUDEP  
Time spent in bed: TIB  
Total sleep time: TST  
Wake after sleep onset: WASO

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### **Competing Interest Statement**

The authors declare no competing interest related to this work

## **1. Introduction**

Obstructive Sleep Apnea (OSA) is a respiratory disorder characterized by recurrent episodes of complete (apnea) or partial (hypopnea) pharyngeal collapse during sleep. These disturbances in respiratory flow can lead to sleep fragmentation (arousals) and/or hypoxemia (Dempsey et al., 2010). Ventilatory recovery from obstructive apnea is a physiological mechanism based on the integration of signals (hypoxemia, hypercapnia, respiratory effort) at the brainstem level, leading to a gradual arousal response going from a subcortical (or autonomic) response only to a cortical response with various patterns of low and high frequency EEG activities (Sforza et al., 2000). The intensity of arousals is however not as much modulated by the intensity of the obstructive event as by individual factors (Amatoury et al., 2016). The arousal response in the context of OSA could thus be a relevant model to study a potential impairment in brain reactivity to respiratory signals.

OSA is highly prevalent in epilepsy, especially in patients with drug-resistant epilepsy (DRE) (Malow et al., 2000). It might be associated with epilepsy worsening and poor seizure control, potentially due to the enhancement of epileptic activity by sleep fragmentation (Chihorek et al., 2007, Peter-Derex et al., 2020). In addition, it was suggested that severe OSA might be a risk factor for Sudden Unexpected Death in Epilepsy (SUDEP) in patients with DRE (Cheng, 2021). SUDEP typically occur at night in patients with frequent generalized tonic-clonic seizures and lack of nocturnal supervision (Purnell et al., 2018). Its pathophysiological mechanisms may primarily involve a seizure-triggered respiratory dysfunction leading to a post-ictal central apnea, possibly associated with autonomic dysfunction (Ryvlin et al., 2013, Stewart et al., 2017, Vilella et al., 2019). Post-mortem data from patients who died from SUDEP showed alterations of neuronal populations in the medulla with evidence for greater reduction in neuromodulatory neuropeptidergic and monoaminergic, including serotonergic, systems, (Patodia et al., 2021) which are involved both in respiratory nuclei stimulation and in ascending arousing systems (Benarroch, 2019, Sulaman et al., 2023). As observed in preclinical studies, overlapping alteration of respiratory control and arousal regulation, including adenosinergic pathway, may contribute to increased risk of SUDEP in case of nocturnal seizure (Buchanan, 2019, Patodia et al., 2021).

However, whether or not patients with DRE epilepsy might develop alteration of the apnea-related arousing response is unknown. The identification of such alterations would be particularly relevant not only to better understand mechanisms leading to SUDEP, but also in the context of identifying biomarkers associated with SUDEP risk. To address this issue, we compared arousals to respiratory events and non-respiratory arousals between patients with DRE and OSA, patients with drug-sensitive epilepsy (DSE) and OSA and OSA patients without epilepsy. We aimed to investigate (i) the cortical arousability assessed by the several indices including the cortical respiratory arousal index/apnea-hypopnea (AHI) ratio and the respiratory arousal threshold (Edwards et al., 2014) (ii) the cardiac autonomic arousing response to respiratory and non-respiratory events, assessed by the heart rate variability (HRV). We hypothesized that patients with epilepsy would have impaired cortical and/or autonomic arousal response evidencing a blunted reactivity during sleep, and that these alterations

would be more pronounced in DRE than in DSE patients, and would be more pronounced for respiratory related than non-respiratory arousals.

## 2. Methods

### 2.1. Patients

We retrospectively reviewed the Center of Sleep Medicine and Respiratory Disease database to identify among patients who had clinical evaluation and a full night polysomnography (PSG), those with DRE or DSE (from 01/2017 to 09/2020) as well as those with OSA but without epilepsy (from 05/2019 to 12/2019), who will be used as controls in the present study. Patients' selection was performed using a two-steps process: (i) identification of eligible patients in the database ; (ii) selection of patients among the eligible population to ensure that the three groups (DSE, DRE and controls) will have the same sample size and will be similar for age, gender, Body Mass Index (BMI) and AHI. Considering the exploratory nature of the study, no hypothesis-driven calculation of the sample size could be performed. The criteria used to identify the eligible population were the following:

- Patients with epilepsy:
  - o Inclusion criteria: patients suffering from epilepsy according to the International League Against Epilepsy definition (Fisher et al., 2014) ; age between 18 and 70; mild to severe OSA (mild: AHI=5-14/h; moderate: AHI=15-29/h; severe AHI $\geq$ 30/h);
  - o Exclusion criteria: artifacts or highly abnormal scalp EEG interfering with sleep staging or arousal scoring; comorbid sleep disorders potentially associated with abnormal arousals (parasomnia, sleep related movement disorders); treatment with continuous positive air pressure (CPAP). Patients with vagus nerve stimulation and/or beta-blockers medication were excluded from autonomic arousal responses analyses.

Patients were then classified into two groups, drug-resistant (DRE, “active”) or drug-sensitive (DSE, “controlled”) epilepsy, according to the persistence or not of seizures despite well-conducted anti-seizure medication. This operational definition was used given the suspected deleterious effect of recurrent seizures on brainstem regions involved in both autonomic system regulation and arousal process (Patodia et al., 2021).

- Control subjects:
  - o Inclusion criteria were: age between 18 and 70; mild to severe OSA.
  - o Exclusion criteria were: epilepsy, artifacts interfering with sleep staging or arousal scoring; associated sleep disorders with abnormal arousals (parasomnia, sleep related movement disorders); treatment with CPAP or Oral Appliance Therapy.

### 2.2. Study protocol and data collection

- Clinical data

Clinical data were reviewed from patients' medical file: age, gender, medical history, current treatments, BMI, substance (tobacco, cannabis, alcohol) consumption. As part of routine diagnosis evaluation, patients were asked to fill out the Epworth Sleepiness Scale (ESS), the Beck Depressive Inventory (BDI), and the Pichot fatigue scale.

- Polysomnography recordings

Full night PSG were conducted in the Sleep Medicine and Respiratory Disease Center with Deltamed, Natus® or Micromed® amplifiers. The following signals were recorded: electroencephalogram (Fp2, C4, T4, O2, Fp1, C3, T3, O1, Fz, Cz, Pz), electro-oculogram, chin and tibialis electromyogram, EKG, nasal airflow (nasal pressure and naso-buccal thermistor), pulse oxymetry, microphone, and respiratory efforts (thoracic and abdominal belts).

- Polysomnography scoring

All PSG recordings were reviewed for the purpose of the present study. Sleep scoring was performed manually by two board-certified neurophysiologists (F.R. with countercheck by L.P.D.) according to the American Academy of Sleep Medicine (AASM) scoring rules (Berry R. B. et al., 2017). Arousals were defined as “an abrupt shift in EEG frequency, which may include theta, alpha and /or frequencies greater than 16 Hz, but not spindles, that last at least 3 seconds, with at least 10 seconds of stable sleep preceding the change”. Scoring of arousals during stage R required “a concurrent increase in EMG lasting at least 1 second ” (ASDA, 1992, Berry Richard B. et al., 2017). Usual sleep parameters were collected: time spent in bed (TIB, min), total sleep time (TST, min), wake after sleep onset (WASO, min), sleep efficiency (SE, %), sleep onset latency (SOL, min), time spent in N3 stage (min, %), time spent in rapid-eye-movement (REM) sleep (min, %). Events scoring allowed to assess the arousal index (ArI, n/h), the respiratory arousal index (Respiratory ArI, n/h), the AHI (n/h), the apnea index (AI) (n/h), the hypopnea index (HI) (n/h), the time spent with SaO<sub>2</sub> < 90% (min), the mean oxygen saturation (%), the nadir oxygen saturation, and the  $\geq 3\%$  oxygen desaturation index (ODI, n/h).

- Cortical arousal response to respiratory events

We hypothesized that patients with epilepsy may show less arousing response to respiratory events, leading to a higher proportion of desaturation-associated versus arousal-associated events, to longer events and to a higher proportion of apnea vs hypopnea. In order to quantify the cortical arousal response to respiratory events, we determined the following indices: respiratory ArI/AHI, ODI/AHI, resp ArI/ODI, IA/IAH, apnea duration. Additionally, an automatic scoring was launched on nasal pressure signal to detect all airflow reductions  $\geq 30\%$  regardless of the presence of an arousal or a desaturation. This detection was performed to overcome the bias linked to the fact that hypopneas are, according to AASM recommendations, only scored if followed by an arousal and/or a  $\geq 3\%$  desaturation (Berry R. B. et al., 2017). As a result, in case of hypo-arousability, one might expect either that hypopneas would be longer and deeper and lead to a desaturation, or that less hypopnea events would be scored. Thus, we extracted the automatic HI. Then, we calculated the (automatic HI – visual HI) / (automatic HI) ratio (expected to be higher if more events have no consequences on cortical arousal or desaturation)

(supplementary figure 1). Last, a respiratory arousal threshold was calculated based on the nadir oxygen saturation (score 1 if  $>82.5\%$ ), the AHI (score 1 if  $<30/h$ ), and the fraction of hypopnea among respiratory events (score 1 if  $>58.3\%$ ) (Edwards et al., 2014). A score of  $\geq 2$  has been showed to correctly predict low arousal threshold with a sensitivity of 80.4% and a specificity of 88.0% (Edwards et al., 2014).

- Autonomic arousal responses to respiratory and non-respiratory events

R waves were detected from the EKG signal to calculate the RR interval (RRI) signal using a dedicated software (Pichot et al., 2016). Initial automatized extraction of RRI signal from EKG data was applied and subsequently checked by visual inspection. In the presence of undetected isolated QRS, ectopic beats or artifacts, the RRI signal was manually corrected or a cubic spline interpolation was used when manual correction was not possible (Daskalov and Christov, 1997, Pichot et al., 2016). EKG data were eliminated from analysis when uncorrected artifacts or ectopic beats occurred in an interval of less than 15 seconds before and 25 seconds after the EEG arousal onset.

Autonomic cardiac changes over time were studied with RRI response and wavelet analysis applied to the RRI signal. Wavelet transform provides temporally localized information and its ability to study autonomic reactivity had been validated by dual atropine and propranolol blockade (Pichot et al., 1999, Rajendra Acharya et al., 2006). Using the mother function Daubechies 4.0, Wavelet analysis was applied to a 2.4 Hz resampled RRI signal. Fast frequencies in RRI signals were gathered in High Frequency band (HF) ( $ms^2$ , 0.15–0.4 Hz) to assess parasympathetic reactivity and in Low Frequency band (LF) ( $ms^2$ , 0.04–0.15 Hz) which is controlled by both the sympathetic and parasympathetic systems; the LF/HF ratio was used to assess sympatho-vagal balance (Pagani et al., 1997). Frequency analyses were chosen for our study as they allow to explore the autonomous nervous system over short duration segments. Then, for each of these calculated autonomic indices (RRI, LF, HF and LF/HF), all 30 s sequences starting  $-10$  s before the onset of arousals and ending  $+20$  s afterward were selected if no additional events were scored in the 20 s period surrounding the considered arousal. From these sequences, the change in the indices was calculated as the difference between each value and the baseline, defined as the mean value from  $-10$  s to  $-1$  s before the arousal. Finally, RRI, HF, LF and LF/HF ratio values were normalized in percentage of the baseline period values to correct for interindividual variability. So, each value is expressed as a percentage of the basal level of each arousal and each RRI variability parameters is presented as mean  $\pm$  standard deviation (SD). The RR data are also presented in absolute values, supplementing the interpretation of normalized data (see supplementary Figure 3).

Four patients in the DRE groups had focal seizures during the recorded night. For these patients, the peri-seizure time windows ( $-5$  min to  $+5$  min) were removed from analyses.

### ***2.3. Statistical analysis***



Quantitative variables were described by the mean (SD) and median (first and third quartiles) depending on the normality of the distribution (Shapiro-Wilk test); qualitative variables were described by the frequency and percentage of each modality (excluding missing data from percentages). Comparison of results between the three groups (DRE, DSE, and controls) were performed with a one-way analysis of variance (ANOVA) or a Kruskal-Wallis test for quantitative results, and the chi-square or Fisher's tests for qualitative results.

RRI variability data were analyzed using Statview® software (SAS Institute, Inc., Cary NC, USA). RRI over 10 seconds pre and 15 seconds after arousal onset were submitted to a two-sided repeated-measures ANOVA. This analysis was calculated with one within-factor time (10 s before and 15 s after arousal onset), and two between factors: arousal type (respiratory and non-respiratory arousals), and population (DES, DRE and control patients). Maximum and latencies to the maximum of RRI changes were studied using an ANOVA with two between factor: type of arousal (respiratory and non-respiratory arousals), and population (DES, DRE and control patients). As changes in RRI variability parameters can be slower than RRI variations, changes of HF, LF, and LF/HF ratio over 10 seconds pre- and 20 seconds after arousal onset were therefore submitted to a two-sided repeated-measures ANOVA with the same within and between factors. Newman-Keuls post hoc test was performed when appropriate. P-values were considered as significant at  $p < 0.05$  for all statistics.

#### ***2.4. Standard Protocol Approvals, Registrations, and Patient Consents***

The study was approved by the Hospices Civils de Lyon Ethics Review Board (N°21\_253, 07/2021). All patients granted informed consent for the use of their data for this study.

### **3. Results**

#### ***3.1. Patients***

Among the 196 patients with epilepsy who had undergone clinical evaluation and a full night PSG, 108 met the eligibility criteria, 33 with DRE and 72 with DES. The need to adjust groups for age, gender, BMI and AHI led us to include 20 DRE and 20 DES. We selected 20 among 179 patients with OSA and without epilepsy to match with the population of patients with epilepsy for age, gender, BMI and AHI; these OSA patients were considered as controls (a flow chart is provided in supplementary figure 2). The clinical data including results of questionnaires are provided in Table 1 and epilepsy-related characteristics are summarized in Table 2. Subjective sleepiness as assessed by ESS was higher in controls than in DSE patients. To note, no statistical difference was found for the number of antiseizure medication between DSE and DRE patients (Chi2,  $p=0.3224$ ).

#### ***3.2. Polysomnography characteristics***

The polysomnographic characteristics are summarized in Table 3. The three groups (controls, DSE and DRE) did not differ significantly for any of the variables except for a lower REM sleep proportion in DSE than in control subjects (17.2 (7.2) % vs 22.0 (6.2) %,  $p=0.05$ ).

### **3.3. Arousability**

#### *3.3.1. Polysomnography-related indices*

No difference was found between the three groups for indices exploring arousability, including the proportion of apnea (10 to 20%) or arousing events (~70% in the three groups) vs events with desaturation (30 to 50% in the three groups) (Table 4). As expected, the automatic HI was higher than the visual HI as it included “innocent” events (without desaturation nor arousal), but in the same proportion in the three groups. The respiratory arousal threshold was estimated as low in the majority of patients in the 3 groups.

#### *3.3.2 Autonomic arousals*

For the autonomic response evaluation, additional exclusion criteria were applied (vagus nerve stimulation, beta-blockers medication and poor-quality EKG signal, see Supplementary figure 2), leaving 17 DRE, 18 DSE and 20 controls in the analysis. A total of 1970 respiratory and 763 non-respiratory arousals were examined in the DRE group, 2148 respiratory and 714 non-respiratory within the DSE group, along with and 2206 respiratory arousals and 778 of non-respiratory arousals in the control group.

Analysis of the cardiac reactivity revealed a significant RRI decrease at the arousal onset ( $(F_{(24, 54)}) = 184.45$ ,  $p < 0.001$ , Figures 1A and 1B). Post-hoc analysis confirmed this decrease in RRI between 1 and 8 seconds after arousal onset in comparison with baseline ( $p < 0.05$ ). These changes were more pronounced in respiratory than in non-respiratory arousals ( $(F_{(1,54)}) = 22.71$ ,  $p < 0.001$ , interaction time x arousal type ( $(F_{(24,54)}) = 9.46$ ,  $p < 0.001$ ), and lower in DRE than in both other groups ( $(F_{(2,54)}) = 3.71$ ,  $p < 0.029$ , interaction time x population:  $(F_{(48,54)}) = 3.09$ ,  $p < 0.001$ , post-hoc tests:  $p < 0.050$ ). Consistently, a lower change in the maximal amplitude of the RRI decrease was observed in DRE compared to two other groups (ANOVA:  $(F_{(2,54)}) = 9.97$ ,  $p = 0.001$ , post-hoc tests:  $p < 0.050$ ) and in non-respiratory compared to respiratory arousals (ANOVA:  $(F_{(1,54)}) = 13.90$ ,  $p = 0.003$  Figure 1C). No difference in the latency of the maximal RRI decrease appeared according to the type of arousal (ANOVA:  $(F_{(1,54)}) = 1.60$ ,  $p = 0.209$ ) and the population (ANOVA:  $(F_{(2,54)}) = 2.20$ ,  $p = 0.116$ , Figure 1D) was observed. Finally, RRI changes in absolute value did not reveal any significant change depending on arousal type (ANOVA:  $(F_{(1, 54)}) = 0.14$ ,  $p = 0.714$ ) but according to groups where RR intervals differed between the three groups, being shorter in patients with epilepsy than in controls and in DSE than in DRE (ANOVA:  $F_{(2, 54)} = 7.23$ ,  $p = 0.001$ , post-hoc tests:  $p < 0.050$ ). Type of arousal and groups still interacted with time (type of arousal:  $F_{(1, 54)} = 8.78$ ,  $p < 0.001$ ; groups:  $F_{(2, 54)} = 2.75$ ,  $p < 0.001$ , supplementary Figure 3)

Regarding RRI variability parameters, we observed a HF power increase ( $(F_{(29, 54)}) = 24.21, p < 0.001$ ) and post-hoc analysis revealed HF increase between 12 and 16 seconds after arousal onset ( $p < 0.05$ , Figure 2A). This increase did not differ according to respiratory and non-respiratory arousals ( $(F_{(1,54)}) = 0.42, p = 0.520$ , interaction time x arousal type ( $(F_{(1,54)}) = 1.22, p = 0.195$ ), but HF increase was higher in DRE compared to the two other groups ( $(F_{(2,54)}) = 1.65, p = 0.197$ , interaction time x population:  $(F_{(2,54)}) = 1.36, p = 0.036$ , post-hoc:  $p < 0.050$ ). LF also increased after arousal onset ( $(F_{(29, 54)}) = 137.34, p < 0.001$ ), with a significant increase between 4 and 20 seconds (post-hoc test:  $p < 0.050$ ). These changes were higher in DRE than in other groups ( $(F_{(2,54)}) = 1.69, p = 0.190$ , interaction time x population:  $(F_{(48,54)}) = 1.52, p = 0.007$ , post-hoc tests:  $p < 0.05$ ) and in respiratory vs non-respiratory arousals ( $(F_{(1,54)}) = 6.65, p = 0.011$ , interaction time x arousal type ( $(F_{(24,54)}) = 6.16, p < 0.001$ ). Finally, LF/HF ratio also increased ( $(F_{(29, 54)}) = 39.75, p < 0.001$ ), with a significant increase between 6 and 12 seconds after arousal onset ( $p < 0.05$ , Figure 2C). This increase did not differ between respiratory and non-respiratory arousals ( $(F_{(1,54)}) = 2.81, p = 0.097$ , interaction time x arousal type ( $(F_{(1,54)}) = 1.15, p = 0.264$ ), but differed between the three groups, being higher in DSE patients than in the 2 other groups and in DRE than in controls ( $(F_{(2,54)}) = 3.99, p = 0.021$ , interaction time x population:  $(F_{(2,54)}) = 1.84, p = 0.001$ , post-hoc tests:  $p < 0.05$ ).

To take into account potential long-term post-ictal autonomic disturbances (Toth et al., 2010), sensitivity analyses were performed excluding the 2 patients who had numerous ( $> 10$ ) seizures during the night and keeping only the first part of the night (before the 1<sup>st</sup> seizure) in the 2 patients who had 2 seizures/night (all in the DRE group) (Supplementary figure 4). This led to remove 353 respiratory and 253 non-respiratory arousals. Regarding RRI decrease, the same results were obtained, except that the latency of the maximal RRI decrease was greater in DRE compared to controls (ANOVA:  $(F_{(2, 52)}) = 3.22, p = 0.040$ , post-hoc tests:  $p < 0.050$ ). However, frequency domain analyses showed several differences. The increase in HF remained significant ( $(F_{(29, 52)}) = 20.61, p < 0.001$ ) but did not differ according to arousals type ( $(F_{(1, 52)}) = 0.38, p = 0.536$ , interaction time x arousal type ( $(F_{(1, 52)}) = 1.12, p = 0.305$ ), nor groups ( $(F_{(2, 52)}) = 0.92, p = 0.400$ , interaction time x population:  $(F_{(2, 52)}) = 0.81, p = 0.854$ ). The increase in LF also remained significant ( $(F_{(29, 52)}) = 31.45, p < 0.001$ ) but without effect of the arousal type ( $(F_{(1, 52)}) = 0.99, p = 0.896$ , interaction time x arousal type ( $(F_{(24, 52)}) = 0.55, p = 0.797$ ) nor the groups ( $(F_{(2, 52)}) = 0.11, p = 0.896$ , interaction time x population:  $(F_{(48, 52)}) = 0.03, p = 0.977$ ). Only the LF/HF ratio increase ( $(F_{(29, 52)}) = 35.53, p < 0.001$ ) differed between the three groups, being higher in DSE patients than in the 2 other groups and in DRE than in controls ( $(F_{(2, 52)}) = 3.92, p = 0.023$ , interaction time x population:  $(F_{(2, 52)}) = 2.01, p < 0.001$ , post-hoc tests:  $p < 0.05$ ), while no difference was observed according to arousal type ( $(F_{(1, 52)}) = 2.03, p = 0.157$ , nor interaction time x arousal type ( $(F_{(1, 52)}) = 0.75, p = 0.831$ ).

#### 4. Discussion

Using PSG recordings in OSA patients with and without epilepsy to investigate cortical and autonomic arousal responses to respiratory events, we found (1) no difference between groups in cortical arousal threshold and (2) a lower autonomic arousal response in DRE vs DSE and controls, evidenced by a lower RRI decrease at arousal onset associated with a higher parasympathetic response while both DRE and DSE patients showed an increased early sympathetic response as compared to controls.

The three groups did not differ for demographic, clinical and polysomnography parameters except for a lower ESS score and lower REM sleep proportions in DSE than in controls. In a recent meta-analysis, subjective sleepiness was not different between patients with epilepsy and controls (Bergmann et al., 2021). However, objective sleepiness as assessed by the multiple sleep latency test was shown to be high in epilepsy patients, with abnormal mean sleep latency (<8 min) in almost 50% of patients whereas only one fourth had elevated score on ESS, suggesting possible sleepiness misperception in this context (Grigg-Damberger et al., 2020). Regarding objective sleep parameters, the increase in wake after sleep onset and REM sleep disturbances (increased latency and decreased quality) are the strongest and more consistent features in epilepsy (Bazil et al., 2000, Sudbrack-Oliveira et al., 2019). These disturbances are particularly pronounced during nights with seizure; this may explain, altogether with a lack of power due to the low size of our population, that we found only a slight decrease in the proportion of REM sleep as only four patients had seizures during the PSG night (Bazil et al., 2000).

The respiratory cortical arousal response was investigated using several indices measuring the proportion of respiratory events or decrease in airflow leading to an arousal (vs a desaturation or no consequence), as well as the intensity of these events (hypopnea vs apnea) and their duration. Additionally, we computed the respiratory arousal threshold as described by Edwards et al (Edwards et al., 2014). None of these parameters differed between the three groups, suggesting that the cortical arousal response is preserved in patient with epilepsy, despite potentially sedative antiseizure medication (Berry et al., 1992, Liguori et al., 2021). However, it is important to note that patients with OSA have an increased arousal threshold, which tends to normalize under CPAP treatment (Haba-Rubio et al., 2005). This may have reduced the difference between patients and controls, and prevented us from demonstrating an elevated arousal threshold in epilepsy. Additionally, our exploration of cortical arousal was binary (arousal vs no arousal) while more subtle alterations might be present. Recently, a quantification of arousal intensity by wavelet transform of the EEG signal was proposed (Azarbarzin et al., 2014). Arousal intensity was thus shown to be a distinct and stable individual trait in OSA patients and healthy individuals, and was not associated with the magnitude of the arousing stimulus (Amatoury et al., 2016). We were not able to conduct such spectral analyses due to the presence of EEG abnormalities in patients with epilepsy, enhanced by the well-known activation of epileptic activity during arousals (Peter-Derex et al., 2020). However, as arousal intensity was demonstrated to correlate with arousal-related tachycardia, investigating autonomic response seemed to be a fair approach to explore arousing systems in this context (Azarbarzin et al., 2014).

Interestingly, the analysis of autonomic system showed significant differences between the three groups with a lower heart rate increase during arousals associated with increased late parasympathetic activation in patients with DRE, while both DRE and DSE groups showed increased sympathetic activation versus controls. Overall, we found that the maximal amplitude of the RRI decrease was higher for respiratory (i.e. life-threatening events) vs non-respiratory arousals, which suggests that the nature of the arousal-inducing stimulus may to some extent influence the intensity of arousal (Peter-Derex et al., 2015). The physiological autonomic response to respiratory events consists in an early cardiac parasympathetic dominance during apnea or hypopnea event, followed by a drop in parasympathetic tone and an increase in sympathetic activation at the end of the event, more pronounced in the presence of a cortical arousal or a deep oxygen desaturation (Chouchou et al., 2014, Guilleminault et al., 2005). This response is thought to result from several peripheral afferent information associated with apnea, such as hypoxia, hypercapnia, acidosis, as well as changes in arterial pressure (baroreflex loop response) and in intrathoracic pressure with activation of lung stretch receptors (Smith et al., 1998). This information is integrated at the brainstem level in several nucleus including the rostral ventrolateral medulla containing pre-sympathetic neurons, and nuclei forming the respiratory central pattern generator which also indirectly contributes to sympathetic activation (Guyenet, 2014). Other subcortical structures (such as the midbrain periaqueducal gray) and the cortical control of ventilation (premotor cortex and supplementary motor area) may also be engaged in breathing recovery in case of arousal/awakening (Faull et al., 2019, Macefield and Gandevia, 1991). In patients with epilepsy, acute seizure-induced cardiorespiratory and autonomic dysfunction have been reported, such as ictal tachycardia (or, less frequent, bradycardia) and ictal hypopnea/apnea, especially during convulsive seizures (Sivathamboo et al., 2020). The autonomic deregulation following convulsive seizures might involve decreased sympathetic tone and possibly increased parasympathetic tone during post-ictal EEG depression, this later finding being debated (Lamberts et al., 2013, Poh et al., 2012). Not only ictal but also interictal autonomic imbalance was reported in patients with epilepsy, with decreased HRV suggesting a shift toward sympathetic dominance, more pronounced in temporal lobe epilepsy and in DRE and progressing over time, although conflicting results have been reported, potentially due to heterogeneous methodological approaches (Myers et al., 2018b, Sivakumar et al., 2016). Recently, four studies investigated autonomic dysfunction as assessed by HRV measured in EEG recordings in patients who had died from SUDEP. The first one found lower HRV during wake and abnormal sleep/wake ratio in patients with vs without SUDEP; to note, the SUDEP group was composed of patients with sodium channel mutation (Myers et al., 2018a). The three other studies found no baseline differences in HRV between the SUDEP group and a control group of living epilepsy patients or healthy individuals (Odom and Bateman, 2018, Surges et al., 2009, Szurhaj et al., 2021). Thus, in the recent meta-analysis investigating changes in HRV parameters in patients with SUDEP (N=72) vs controls, no significant difference in time and frequency domains parameters were found, although a trend toward an increased LF/HF ratio both in wake and sleep states was observed (Evangelista et al., 2023). Importantly, Szurhaj

et al. reported that patients with SUDEP had lower cardiac autonomic stimulation induced by hyperventilation than living patients with epilepsy, suggesting lower sympathetic reactivity (Szurhaj et al., 2021). The mechanisms linking possible autonomic dysfunction and increased SUDEP risk are not fully understood, but might involve a chronic deregulation and an acute seizure-related dysfunction leading to SUDEP, keeping in mind that the pathological processes might be pluri-factorial and not homogenous from one patient to another (Myers et al., 2018a, Odom and Bateman, 2018). This dysregulation might result from the alteration of neuronal networks involved in both autonomic system regulation and arousal process, possibly due to seizure-induced brain injury (Patodia et al., 2021).

We acknowledge several limitations to this study. First, the influence of antiseizure medication on arousal response and on HRV cannot be ruled out as, even if the number of drugs did not differ between DRE and DSE patients, this confounding factor was obviously present for the comparison with the control group. Second, the small size of our sample did not allow us to perform subgroup analyses, regarding the epileptic syndrome. In particular, four patients had generalized epilepsy in the DSE group. Although previous studies reported similar alterations of HRV in patients with focal epilepsy and those with primary generalized epilepsy (Mazzola and Rheims, 2021, Tomson et al., 1998), with the exception of the specific situation of Dravet Syndrome (Myers et al., 2018a), we cannot formally exclude that arousal reactivity might differ across epilepsy syndrome. Fourth, our sensitivity analyses excluding patients with seizures during the PSG night confirmed the lower (and delayed) decrease in RRI during arousals in DRE and the higher increase in LF/HF ratio in DRE and DSE but more pronounced in the latter group suggesting an increase in sympathetic tone vs controls in both groups but a relative increase in parasympathetic tone in DRE vs DSE. However, results regarding LF and HF were not significant any more, likely due to a lack of power. This stresses the need for further studies comparing nights with and without seizures, and controlling for clinical and electrographic seizures the previous day. Finally, although OSA is highly prevalent in patients with epilepsy, our results might not apply to epilepsy patients without OSA.

## **Conclusion**

In this study, we explored specifically arousal response to respiratory events during sleep in patients with epilepsy. Using OSA as a model of respiratory dysfunction during sleep, we were able to demonstrate arousal-related autonomic dysfunction in patients with DRE, highlighting the added value of autonomic stimulation paradigms vs baseline interictal recordings to uncover subtle alterations in this context (Szurhaj et al., 2021). In addition to existing literature, our findings suggest that baseline sympatho-vagal imbalance in DRE patients might be associated with a lower capacity to react to stressful stimulations. During sleep, this impaired cardiac response to sympathetic stimulation might be amplified by arousal system dysfunction and possibly OSA. Indeed, OSA is associated with decreased HRV during wakefulness in patients without epilepsy (Roche et al., 1999), and might be a risk factor for SUDEP although this remains debated (Cheng, 2021, McCarter et al., 2018, Phabphal et al., 2021).

Whether the impaired apnea-induced autonomic arousal that we observed in patients with DRE might be associated with risk of post-ictal respiratory dysfunction and, eventually, with long-term risk of SUDEP, will have to be investigated. Further study investigating the impact of OSA treatment on autonomic function in OSA patients with DRE would also be of high interest.

### **Data availability statement**

The data for this project are available upon reasonable request to the senior author and in compliance with ethics. The software used to process EKG data can be downloaded at <https://anslabtools.univ-st-etienne.fr/en/index.html> (Pichot *et al.*, 2016).

### **Conflict of interest: none**

**Author contribution:** Design of the study: LPD, SR and FR; Data collection: LPD, FR, TP, FG, HB, EC, PLC, ES ; Data analysis: FR, FC, VP, SR, LPD; Results interpretation: FR, FC, VP, FR, SR, LPD; Manuscript drafting and reviewing: FR, FC, VP, FR, TP, FG, HB, EC, PLC, ES, SR, LPD

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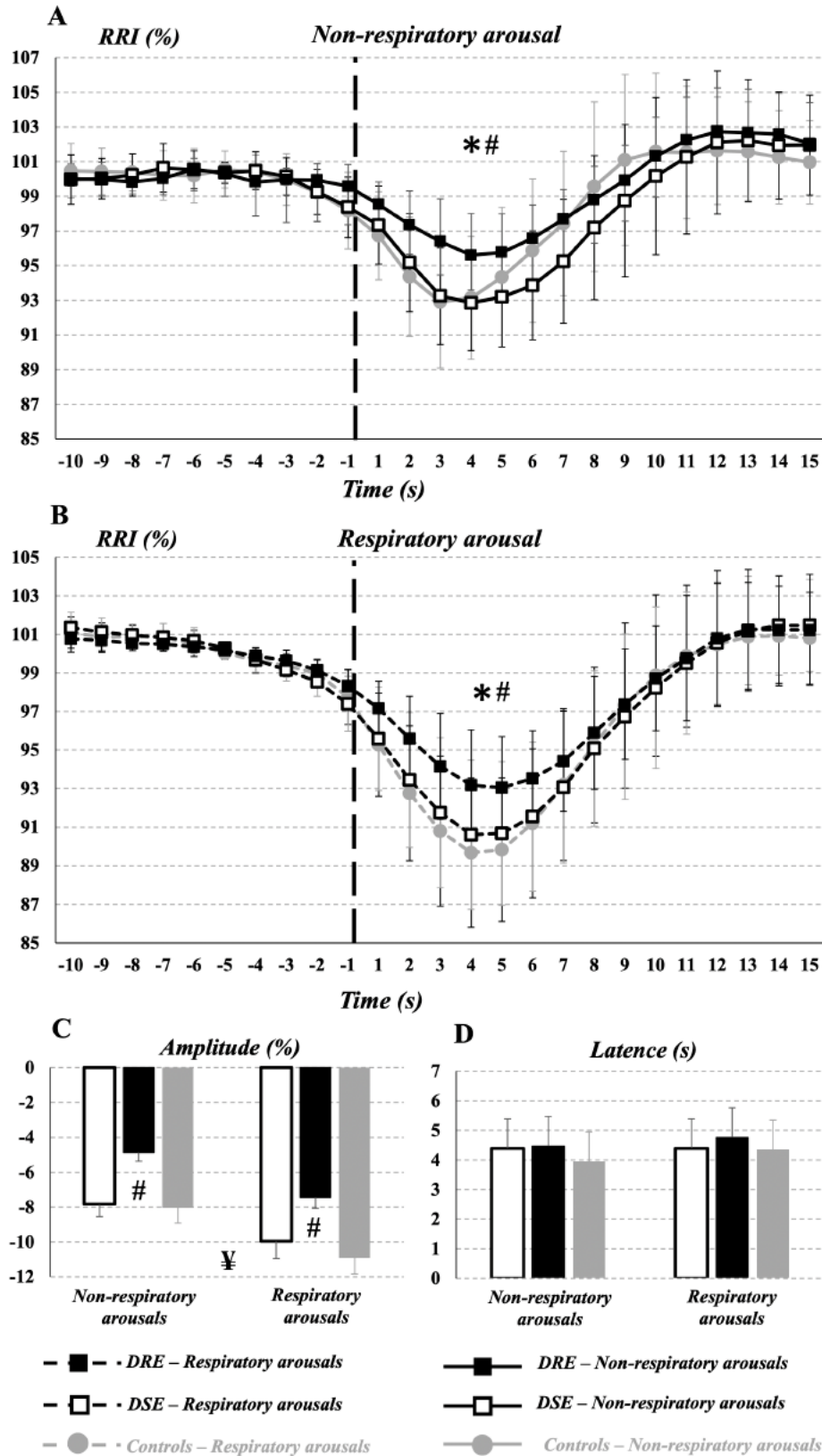
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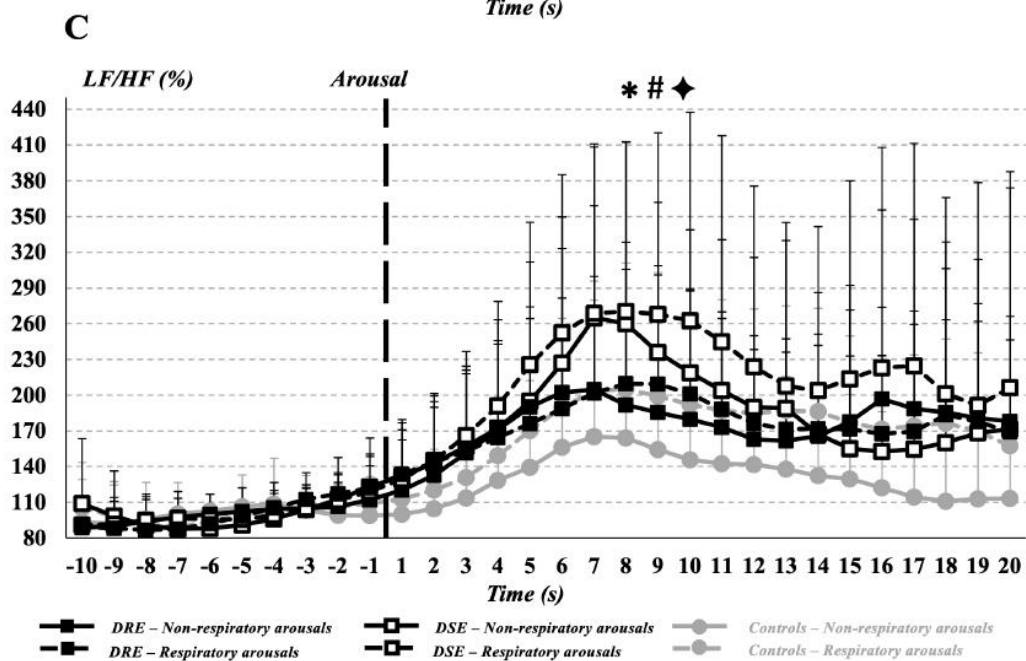
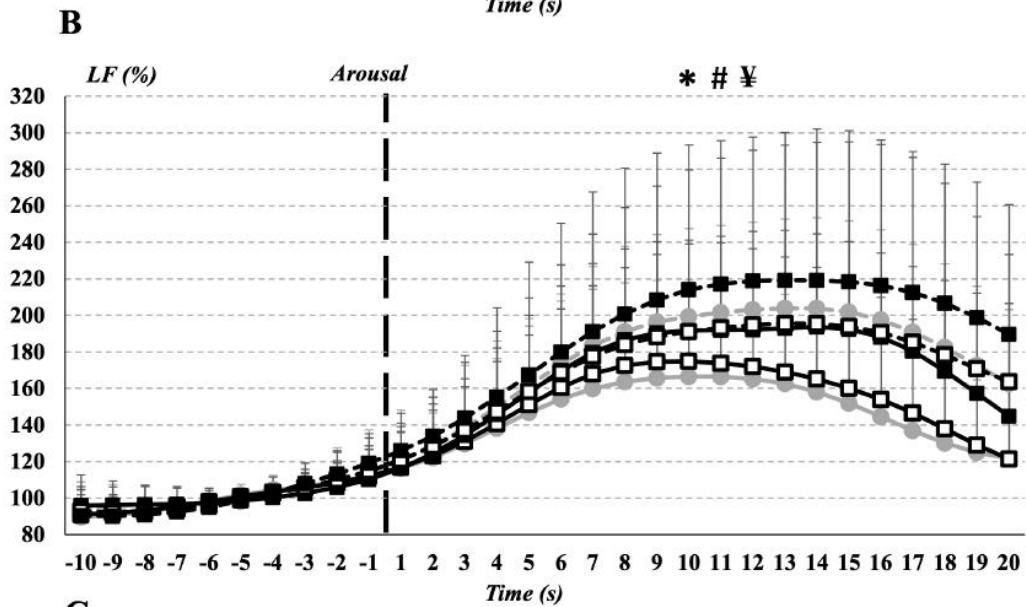
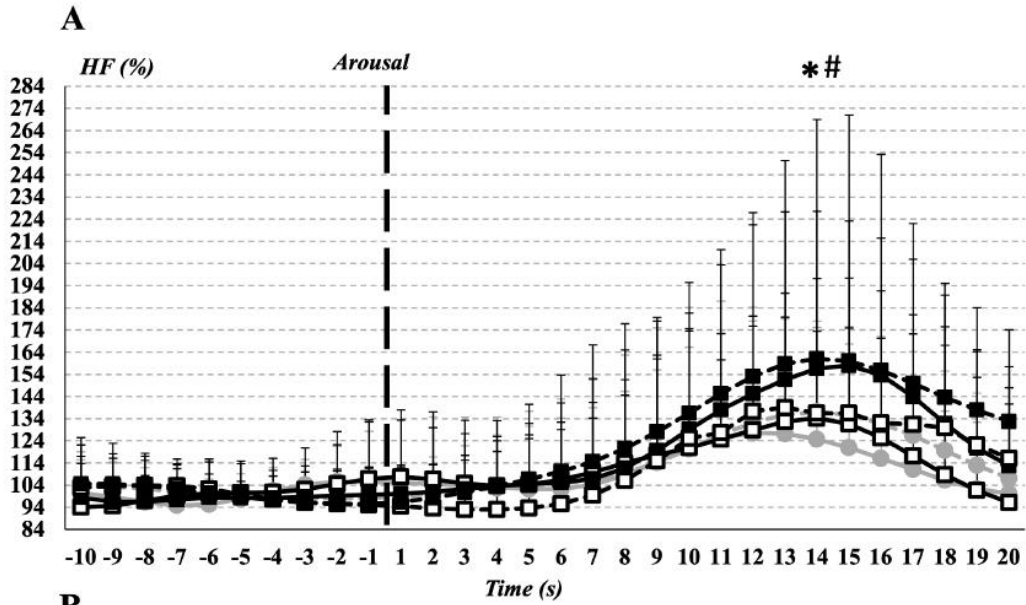
**Figure captions (all figures are in black and white)**

**Figure 1. Cardiac reactivity to respiratory and non-respiratory arousals.** Time course of RR intervals (RRI, mean and standard deviation) during (A) non-respiratory and (B) respiratory arousals; (C) amplitude of RRI decrease; and (D) latency to the peak of the RRI decrease. RRI decreases between 1 and 8 seconds after arousal onset in comparison with baseline (\*:  $p < 0.05$ , panels A and B) and drug-resistant epilepsy (DRE) and non-respiratory arousals present lower cardiac reactivity (#:  $p < 0.05$  for DRE vs drug-sensitive epilepsy (DSE) and vs controls, and ¥:  $p < 0.005$  for respiratory vs non-respiratory arousals). Vertical dotted lines symbolize arousal onset.



**Figure 2. Autonomic cardiac reactivity to respiratory and non-respiratory arousals.** Time course of (A) high frequency (HF) power, (B) low frequency (LF) power, and (C) LF/HF ratio variations (mean and standard deviation), from 10 seconds pre- to 20 seconds post-arousal onset. HF increases between 12 and 16 seconds after arousal onset in comparison with baseline (\*:  $p < 0.05$ ) with a higher increase

in DRE compared to DSE and to controls (#:  $p < 0.05$ ), and LF increases between 4 and 20 seconds (\*:  $p < 0.05$ ), with a higher increase in respiratory vs non-respiratory arousals (¥:  $p < 0.05$ ) and in drug-resistant epilepsy (DRE) vs drug-sensitive epilepsy (DSE) and vs controls (#:  $p < 0.05$ ). LF/HF showed a significant increase from baseline (between 6 and 12 seconds, \*:  $p < 0.05$ ), higher in DSE (◆:  $p < 0.05$ ) than both other groups, and in DRE than in controls (#:  $p < 0.05$ ). Vertical dotted lines symbolize arousal onset.



## Tables

**Table 1. Clinical characteristics of the patients**

BMI: body mass index; ESS: Epworth Sleepiness Scale; BDI: Beck Depressive Inventory

N mentioned if < 20 (missing data)

\* post hoc analysis: ESS was higher in controls than in patients with drug-sensitive epilepsy

	<b>Controls (N=20)</b>	<b>Drug-sensitive epilepsy (N=20)</b>	<b>Drug-resistant epilepsy (N=20)</b>	<b>p-value</b>
<b>Age, mean (SD)</b>	47.9 (14.2)	44.4 (12.6)	50.1 (12.7)	0.09
<b>Gender, N (%) F</b>	8 (35%)	5 (25%)	9 (45%)	0.39
<b>Tobacco consumption, N (%)</b>	19 (26%)	5 (25%)	6 (30%)	0.48
<b>Cannabis, N (%)</b>	3 (13%) (N=14)	2 (10%) (N=17)	0 (0%) (N=18)	0.44
<b>BMI, median (Q1-Q3)</b>	25 (21.6-27.1)	27.3 (24.3-35.4)	25.1 (22.7-29.9)	0.35
<b>ESS, mean(SD)</b>	11.5 (5.2)* (N=19)	8.4 (3.7)* (N=14)	9.7 (4.4) (N=15)	0.034
<b>BDI, median (Q1-Q3)</b>	9.0 (5.0-17.0) (N=17)	9.0 (6.0-17.0) (N=15)	12.0 (4.0-13.0) (N=9)	0.59
<b>Pichot Scale, mean(SD)</b>	14.7 (7.6) (N=17)	14.7 (7.9) (N=16)	13.0 (6.1) (N=16)	0.57

**Table 2. Characteristics of epilepsy**

SHE: sleep related hypermotor epilepsy; PSG: polysomnography

	<b>Drug-sensitive epilepsy (N=20)</b>	<b>Drug-resistant epilepsy (N=20)</b>
<b>Epileptic Syndrome, N (%)</b>		
Idiopathic Generalized Epilepsy	2 (10%)	0 (0%)
Generalized Epilepsy of unknown cause	2 (10%)	0 (0%)
Structural focal Epilepsy	11 (55%)	8 (40%)
Focal Epilepsy of Unknown cause /(SHE)	4 (20%)/ 0 (0%)	10 (50%)/ 2 (10%)
Missing data	1 (5%)	0 (0%)
<b>Vagus nerve stimulation, N (%)</b>	0 (0%)	2 (10%)
<b>Duration of epilepsy (years), mean (SD)</b>	21.4 (19.2) (N=19)	27.6(18.3)
<b>Number of antiseizure drugs, N (%)</b>		
0	1 (5%)	1 (5%)
1	6 (30%)	2 (10%)
2	9 (45%)	7 (35%)
≥3	4 (20%)	10 (50%)
<b>Antiseizure medications</b>		
Benzodiazepine	7 (35%)	7 (35%)
Levetiracetam	9 (45%)	3 (15%)
Lamotrigine	5 (25%)	5 (25%)
Sodium Valproate	4 (20%)	3 (15%)
Carbamazepine	4 (20%)	7 (35%)
Lacosamide	3 (15%)	5 (25%)
Topiramate	1 (5%)	4 (20%)
Perampanel	1 (5%)	7 (35%)
Others	2 (10%)	4 (20%)
<b>EEG anomalies during the PSG</b>		
None	9 (45%)	2 (10%)
Seizures during the PSG night (focal)	0 (0%)	4 (20%)
Epileptic discharges during the PSG night	1 (5%)	2 (10%)
Interictal paroxysmal activity during the PSG night	7 (35%)	7 (35%)
Abnormal EEG slowing during the PSG night	3 (15%)	5 (25%)



**Table 3. Polysomnography parameters**

TST: total sleep time; SOL: sleep onset latency; WASO: wake after sleep onset; SE: sleep efficiency; REM: rapid eye movement sleep; AHI: apnea hypopnea index; AI: apnea index; ArI: arousal index; ODI: oxygen desaturation index; min: minutes

\* post hoc analysis: rapid-eye-movement (REM) sleep (%) duration was lower in patient with drug-sensitive epilepsy than in control

N mentioned if < 20 (missing data)

	<b>Controls (N=20)</b>	<b>Drug-sensitive epilepsy (N=20)</b>	<b>Drug-resistant epilepsy (N=20)</b>	<b>p-value</b>
<b>TST (min), mean (SD)</b>	395.1 (73.1)	391.5 (77.7)	413.8 (75.8)	0.61
<b>SOL (min), median (Q1-Q3)</b>	26.5 (11.1-48.6)	18.7 (5.9-44.9)	19.8 (6.4-38.8)	0.65
<b>WASO (min), median (Q1-Q3)</b>	55 (28.3-69.5)	56.5 (34.8-93.1)	76.5 (25.5-102.3)	0.46
<b>N3 (min and %), mean(SD)</b>	93.4 (38.1) min 23.6 (8.3) %	84.9 (38.0) min 21.2 (8.7) %	90.6 (42.8) min 21.6 (9.6) %	0.79 0.66
<b>SE (%), median (Q1-Q3)</b>	0.82 (0.74-0.89)	0.81 (0.71-0.88)	0.79 (0.70-0.90)	0.87
<b>REM (min et %), mean (SD)</b>	87.0 (31.4) min et 22.0 (6.2)* %	70.6 (35.3) min 17.2 (7.2)* %	76.9 (32.3) min 18.3 (5.6) %	0.30 0.05
<b>REM latency, median (Q1-Q3)</b>	91.0 (66.2- 171.5)	115.5 (81.5- 179.0)	103.5 (78.3- 150.1)	0.47
<b>AHI (n/h), median (Q1-Q3)</b>	23.5 (15.8-34.3)	23.8 (11.2-44.1)	18.9 (11.5-29.9)	0.53
<b>AHI category (mild/moderate/severe %)</b>	15%, 45%, 40%	30%, 30%, 40%	30%, 45%, 25%	0.59
<b>AI (median (Q1-Q3))</b>	3.6 (1.0-6.4)	3.0 (1.0-7.3)	2.3 (1-7.6)	0.89
<b>ArI (n/h), median (Q1-Q3)</b>	27.2 (19.5-31.4)	29.5 (13.0-38.1)	22.4 (16.5-29.8)	0.40
<b>Respiratory ArI (n/h), median (Q1-Q3)</b>	15.7 (12.4-22.2)	19.4 (7.3-31.5)	13.7 (9.0-18.8)	0.38
<b>ODI ≥ 3% (n/h), median (Q1- Q3)</b>	13.9 (5.0-19.4) (N=19)	7.0 (1.1-19.9)	4.4 (1.9-15.3)	0.37
<b>Nadir désaturation (%), median (Q1-Q3)</b>	88.0 (85.0-90.0) (N=19)	87.0 (84.0-91.0) (N=19)	89.0 (85.0-91.0)	0.96
<b>Average apnea duration (s), median (Q1-Q3)</b>	18.0 (15.5-20.5)	18.3 (16.1-23.1)	17.7 (15.6-24.7) (N=19)	0.48
<b>HI, automatic detection, mean(sd)</b>	37.4 (16.8)	37.1 (17.1) (N=19)	36.7 (19.2) (N=17)	0.99

**Table 4. Polysomnography-related indices of arousability**

AHI: apnea hypopnea index; AI: apnea index; ArI: arousal index; ODI: oxygen desaturation index; HI: hypopnea index

N mentioned if < 20 (missing data)

	<b>Controls (N=20)</b>	<b>Drug-sensitive epilepsy (N=20)</b>	<b>Drug-resistant epilepsy (N=20)</b>	<b>p-value</b>
<b>ODI/AHI, median (Q1-Q3)</b>	0.5 (0.3-0.7) (N=19)	0.5 (0.1-0.8)	0.3 (0.1-0.5)	0.43
<b>Respiratory ArI/AHI, median (Q1-Q3)</b>	0.7 (0.6-0.9)	0.7 (0.6-0.8)	0.7 (0.5-0.8)	0.81
<b>AI/AHI (median, Q1-Q3)</b>	0.1 (0.0-0.02)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.71
<b>(HI (automatic detection) - HI(visual detection)) / HI (automatic detection) (median, Q1-Q3)</b>	0.3 (0.1-0.6)	0.4 (0.2-0.6) (N=19)	0.5 (0.0-0.7) (N=17)	0.87
<b>Respiratory arousal threshold (high: 0-1 versus low: 2-3)</b>	21% vs 79% (N=19)	16% vs 84% (N=19)	15% vs 85% (N=20)	0.86