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Wavelet analysis of heart rate variability related to nocturnal frontal lobe epilepsy seizures

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1. INTRODUCTION

1. 1. THE CENTRAL AUTONOMIC NETWORK

1.1.1. Epileptic seizures and the central autonomic network

During epileptic seizures various autonomic symptoms (cardiovascular, gastrointestinal, respiratory, urogenital, sexual, cutaneous) frequently occur either as an accompaniment to other seizure symptoms or as the predominant seizure manifestation. These symptoms do not seem to represent simple reactions to motor manifestations of seizures, but are probably the consequence of seizures originating or secondarily involving the cerebral areas of the central autonomic network (CAN) [1-3].

Investigating autonomic symptoms could yield important clinical information on the localization and lateralization of the seizure onset zone and help in the differential diagnosis with other non-epileptic events. It could also serve to clarify the pathophysiology of serious complications of epilepsy such as sudden unexplained death (SUDEP), seizure-induced cardiac arrhythmias or neurogenic pulmonary edema [4].

The present study will focus on the ictal cardiovascular manifestations of nocturnal frontal lobe epilepsy (NFLE), a distinct partial epileptic syndrome whose clinical features comprise a spectrum of paroxysmal motor manifestations of variable duration and complexity, occurring mainly during sleep [5]. Areas of the CAN implicated in cardiovascular manifestations during NFLE seizures will then be reviewed (Figure 1).

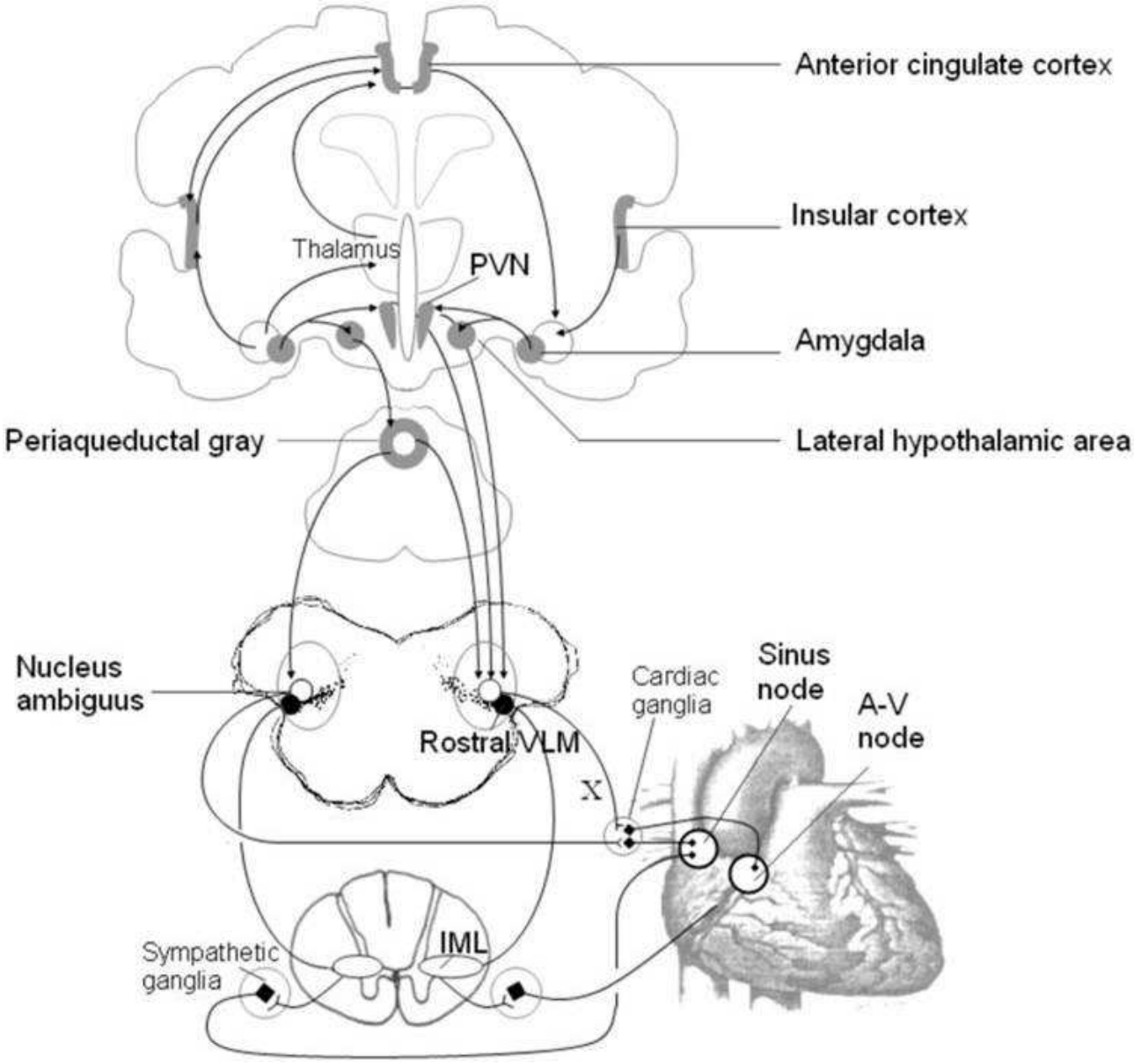

Figure 1. From Britton and Benarroch [6]: Cortical and subcortical structures potentially involved in cardiovascular manifestations during seizures. IML: intermediolateral cell columns; PVN: paraventricular nucleus; VLM: the rostral ventrolateral medulla.

1.1.2. Functional anatomy of the central autonomic network

The CAN comprises several interconnected cortical and subcortical regions devoted to the tonic, reflex and adaptive control of autonomic functions [7]. In particular, the insular cortex, prefrontal cortex (ventromedial prefrontal cortex and anterior cingulate gyrus) and amygdala are involved in modulating cardiac sympathetic and parasympathetic outflow. These areas integrate cardiovascular responses related to behavior and emotion through connections with the following regions:

- The lateral hypothalamic area;
- The periaqueductal gray matter (PAG), the parabrachial region of the dorsolateral pons and the nucleus of the solitary tract (NST) in the brain stem;
- The "effector" regions of the medulla and the spinal cord: the nucleus ambiguous (NA), the rostral ventrolateral medulla (VLM) and the intermediolateral cell columns (IML) [8].

1.1.2.1. The insular cortex and the hypothesis of hemispheric lateralization in cardiovascular control

Neuroanatomical tracer and electrophysiological studies in animals and functional neuroimaging in humans indicate that the insular cortex can be viewed as a primary viscerosensory area receiving gustatory and general visceral afferents. This information reaches the insular cortex through projections from the lamina I of the spinal cord, the NST, the parabrachial nucleus and the lateral hypothalamic area that relay in the parvocellular subdivision of the ventroposterior complex of the thalamus. The insular cortex contains topographically organized representations of taste, pain, temperature, itch and sexual and visceral sensations. In addition, the insular cortex is part of the second

somatosensory area as it receives complex somatosensory inputs from the contralateral body via projections from the parietal lobe [8].

The insular cortex has reciprocal connections with the other areas of the CAN involved in cardiovascular control, the ventromedial prefrontal cortex and anterior cingulate gyrus, and projects to the amygdala, the lateral hypothalamus, and the PAG [8]. Therefore electrical stimulation of the insular cortex elicits changes in heart rate and blood pressure.

There is experimental and clinical evidence that the insular cortex may exert a lateralized influence on cardiovascular autonomic control. In humans, cortical intraoperative stimulation of the left insular cortex elicited heart rate (HR) decrease and hypotension, whereas stimulation of the right insular cortex resulted in tachycardia and hypertension, suggesting a lateralization of parasympathetic activity to the left insular cortex and sympathetic activity to the right [9]. A right insular influence on sympathetic parameters was also documented with FMRI and PET techniques [10-11].

However, studies investigating changes in modulation of the cardiovascular system after stroke have yielded conflicting results with respect to hemispheric lateralization. A reduction in parasympathetic cardiovascular function and an increased risk of complex arrhythmia and sudden death was found in patients after right hemispheric stroke [12-15] while left insular lesions led to increase sympathetic cardiac modulation [16-17] and sudden death [18]. Other studies confirmed the association between cardiac autonomic dysregulation and cerebral infarction irrespective of the side of the lesion [19]. Even studies using unilateral intracarotid amobarbital hemispheric inactivation (Wada test) failed to establish accordance in hemispheric lateralization of sympathetic and vagal modulation of the heart [20-23].

Lastly, an anteroposterior distribution of response within the insular cortex of each side was observed, with an increase in sympathetic parameters more often elicited from the

anterior part of the right insular cortex and bradycardia from the posterior part of the left [9].

1.1.2.2. The prefrontal cortex: the ventromedial prefrontal cortex and the anterior cingulate gyrus

The medial prefrontal cortex is considered a "visceral motor cortex" and includes the ventromedial prefrontal cortex and the anterior cingulate gyrus.

The ventromedial prefrontal cortex is the site of convergence and integration of processed exteroceptive and viscероceptive information and is involved in high level emotional and cognitive functions. In humans lesions of this area abolish autonomic preparatory reactions in response to emotionally significant stimuli [24].

The anterior cingulate gyrus, the "executive" region of the cingulate gyrus, sends projections to the hypothalamus, PAG, parabrachial nucleus, NST, NA and rostral VLM. Bilateral electrical stimulation of the rostral portion of the anterior cingulate cortex, applied before frontal gyrectomy in psychotic patients, elicited autonomic responses including increases or decreases in heart rate, blood pressure and respiratory rate. Complete respiratory arrest was also observed [25]. Moreover bradycardia and asystole was induced in a patient during electrical stimulation of the left cingulate gyrus as part of presurgical evaluation of drug-resistant epilepsy [26]. These autonomic responses were previously obtained during electrical stimulations of the anterior cingulate cortex in animals [27-29].

Neuroimaging studies with PET and fMRI investigated autonomic-related cortical activity with experimental paradigms measuring different parameters of physiological arousals (blood pressure, heart rate, skin conductance activity) in response to several tasks (decision-making, arithmetic and motor tasks). These studies reported increased activity in

the rostral anterior cingulate region and ventral prefrontal cortex to correlate with autonomic measures and suggested the involvement of these regions in the modulation of cardiovascular responses to arousal stimuli, irrespective of the nature of the demands [10, 30-34]. Moreover, patients with focal damage to the anterior cingulate gyrus presented blunted autonomic arousal response to tasks requiring mental stress [33].

A recent study recording directly skin conductance activity and heart rate changes in response to multi-voltage stimulation of the anterior cingulate cortex during stereotactic limbic surgery in psychotic patients found a voltage-response relationship [35]. These data confirmed the crucial role of the anterior cingulate cortex in the neuroanatomical circuitry responsible for autonomic modulation [35].

1.1.2.3. Other cerebral regions involved in cardiovascular modulation

The amygdaloid complex consists of different subnuclei with specific afferent and efferent connections whose function is to interpret the affective significance of incoming sensory information and to generate the appropriate autonomic and behavioral responses. The central nucleus of the amygdala is the primary effector region of this complex and, together with the bed nucleus of the stria terminalis, forms an anatomofunctional unit, “the extended amygdala”, responsible for the autonomic response to emotion [8]. In particular, the amygdala is part of a complex circuit comprising the orbital prefrontal cortex and anterior cingulate gyrus that plays a crucial role in integrated response to aversive stimuli, including the conditioned fear response [36]. Electrical stimulation of the central nucleus of the amygdala in animals resulted in variable blood pressure and heart rate changes [37-38].

The hypothalamus contains several regions controlling autonomic functions, namely the paraventricular nucleus (PVN), dorsomedial nucleus and lateral hypothalamus which are

closely interconnected and project to the brain stem and spinal autonomic nuclei. These areas are involved in integrated autonomic responses to stress and mechanisms of behavioral arousal. The lateral hypothalamic area has a critical role in mediating cardiovascular and other autonomic responses initiated in the insular cortex and amygdala [8]. Electrical stimulation of the lateral hypothalamus in animals induced bradycardia and hypotension, whereas stimulation of medial hypothalamic sites produced a tachycardia/pressor response pattern [39].

1.1.3. Parasympathetic and sympathetic control of the heart

Although cardiac automaticity is intrinsic to various pacemaker tissues, heart rate (HR), excitability and contractility of the heart are largely under the control of parasympathetic and sympathetic autonomic nervous system.

Parasympathetic influence on the heart, mediated by the vagus nerve and acetylcholine release, leads to a decrease in HR, atrio-ventricular (AV) conduction and ventricular excitability through activation of muscarinic receptors. In particular, the vagus exerts a beat-to-beat control of HR that depends largely on the level of innervation of the sinus atrial node (SA).

The sympathetic control of the heart, mediated by norepinephrine acting primarily on β_1 receptors, produces increases in HR, AV conduction and ventricular excitability and contractility [40].

The parasympathetic influence on the heart arises primarily from the NA with contributions from the dorsal motor nucleus of the vagus. The NA comprises two functional regions: the dorsal NA that contains brachimotor neurons innervating the palate, pharynx, oesophagus and larynx respectively and the ventral NA where the preganglionic neurons innervating the heart are located [41].

Sympathetic innervation of the heart arises from the preganglionic neurons in the IML columns in the upper thoracic segments of the spinal cord. The sympathetic influence on the heart is lateralized, as the right sympathetic ganglia predominantly innervate the SA node and increase HR, while the left ganglia innervate the AV node and ventricles, increasing AV conduction, ventricular excitability and cardiac contractility [40]. Balance of parasympathetic and sympathetic modulation is critical for control of cardiac function and is regulated by two main influences [42]:

1. **Medullary reflexes** triggered by activation of baroreceptors, cardiac receptors and chemoreceptors integrated at the level of the NST and in the rostral VLM;

2. **Descending influences** from the cerebral cortex, amygdala, hypothalamus and PAG mediating integrated responses to internal or external stressors, in part by affecting the gain of medullary reflexes.

The major excitatory effect on cardiovagal activity is due to baroreceptor inputs via a relay in the NST. Cardiovagal neurons are otherwise inhibited by inputs from the central inspiratory generators and lung inflation and by the stimulation of the hypothalamic "defense" area. Respiration also modulates the basic discharge of cardiovagal motoneurons presenting variations in their basal potentials coupling the respiratory cycle, with hyperpolarization during inspiration and depolarization postinspiration [43]. Moreover, the excitatory influence of baroreceptor on cardiovagal neurons is decreased during inspiration. This strong respiratory modulation of cardiovagal activity is the main determinant of the respiratory sinus arrhythmia (tachycardia in inspiration, bradycardia in expiration) observed in physiological conditions.

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1.2. HEART RATE VARIABILITY

1.2.1. Definition of heart rate variability

Heart rate variability (HRV) refers to the beat-to-beat variations in HR, expressed by the interval between R waves of two consecutive QRS complexes (RRi). HRV is a typical feature of physiological autonomic control of the heart and reflects spontaneous fluctuations of cardiovagal and sympathetic nerve activity. Under resting conditions, the ECG of healthy individuals exhibits periodic RRi changes according to the respiratory cycle (RRi decreases during inspiration and increases during expiration). This rhythmic phenomenon, known as respiratory sinus arrhythmia, depends primarily on inhibition of cardiovagal neurons during inspiration by the central respiratory generator and pulmonary afferents. However HR variance in normal subjects also depends on the non respiratory mechanism of control of parasympathetic and sympathetic activity, like thermoregulatory and blood pressure influences [1-3].

Spectral analysis of HRV is routinely used and recognized for the assessment of autonomic control of the heart in healthy subjects and various pathological conditions. It is widely accepted that HRV comprises three major components: a very low frequency component (VLF: 0÷0.04 Hz); a low frequency component (LF: 0.04÷0.15 Hz) and a high frequency component (HF: 0.15÷0.4 Hz) [4].

The HF component has been related mainly to parasympathetic outflow and respiratory rhythm [5-7], while the significance of LF component is more controversial. Some believe it to be an index of sympathetic activity [7, 8], while others consider it an indicator of both sympathetic and parasympathetic influences [5, 9]. The LF/HF ratio is however widely used as an indicator of the so-called sympathovagal balance, with high values indicating an

autonomic shift toward sympathetic activity. Physiological correlates of VLF components are not well understood.

The measurement of VLF, LF, and HF power components is usually made in absolute values of power (milliseconds squared). However LF and HF could also be measured as normalized units representing the percentage of each power component in proportion to the total power except for the VLF component.

The normalized indices are used to minimize any changes in the absolute magnitude of total power of HRV on the values of LF and HF components that could occur for example in some conditions associated with sympathetic activation, and to better represent the modulation exerted by the two branches of the autonomic nervous system. Nevertheless, for a complete description of the power distribution in spectral components it is preferable to quote normalized units together with absolute values of the LF and HF power [1].

Spectral analysis of 24-hour recordings shows that LF and HF expressed in normalized units exhibit a circadian pattern and reciprocal fluctuations in normal subjects, with higher values of LF in the daytime and of HF at night [7, 10]. Changes in LF/HF ratio have been observed among sleep phases with a predominance of parasympathetic activity during NREM sleep (decrease of LF/HF ratio) and an increase in LF/HF during REM sleep [11].

HRV also varies across ages with a reduction of overall HRV and high-frequency fluctuations with advanced age [12]. In addition a sex difference in HRV has been reported with relatively greater high-frequency variability in healthy woman compared to men across all ages [13].

1.2.2. Spectral analysis of HRV

Different mathematical approaches have been applied for HRV analysis [14]. Among these, Fast Fourier Transform (FFT) and autoregressive spectral analysis are the most

frequently used. FFT decomposes each signal into a series of sinusoidal functions of different frequencies and amplitudes leading to the definition of a frequency spectrum of the signal. The application of FFT to the analysis of HRV in humans disclosed the three components of HRV described above (VLF, LF, and HF).

However, FFT has important limitations making it unsuitable for the analysis of short and transient changes in HRV. FFT is appropriate for signals whose frequency contents do not change in time (stationary signals), while almost all biological signals are not stationary (EEG, ECG). Moreover it requires a long period of recording as at least five minutes are recommended for the optimal measurement and interpretation of short-term HRV. Finally FFT gives information about the frequency components of the signal, but it does not indicate when a particular frequency occurs.

Wavelet transform (WT) was introduced in medicine as a signal-analysis technique to overcome the limits of FFT. Like FFT, WT is a linear signal transformation made by decomposing the signal into a group of basic functions (WT frame) that are scaled versions (stretched or compressed) of the same prototype, called the mother function (MF). The analysis consists of sliding a window of different weights (corresponding to different levels and frequency bands) containing the WT function throughout the signal. The first levels (2, 4, 8, ...) correspond to a wavelet analysis conducted with a small value of the dilatation factor, thus representing high-frequency variations in the signal. On the contrary, the last levels (..., 32, 64, 128….) correspond to a wavelet analysis conducted with a large value of the dilatation factor, thus representing low-frequency variations in the signal. The evolution of each frequency band, composing the initial signal, could be followed through time. Finally WT gives a list of coefficients which measure the correlation between the initial signal and the wavelet function at each level.

Therefore WT allows a sliding temporally localized analysis of the signal providing concomitant time and frequency information. Moreover, the shape of the analyzing equation of WT in not fixed as the sinusoidal shape of FFT, and can be chosen to better fit the shape of the analyzed signal. Thus, WT is suitable for processing non-stationary signals and may assess the sudden and transient changes in sympathovagal balance occurring in different clinical situations [15-17].

1.2.3. Application of WT analysis of HRV in sleep medicine

Wavelet transform has been applied to evaluate the diagnostic value of HRV changes in different sleep-related pathological conditions like sleep fragmentation and obstructive sleep apnoea syndrome (OSAS) and to define time-dependent oscillations in sympathetic and parasympathetic activity associated with motor events arising from sleep.

Sleep fragmentation, a common feature of sleep disorders determining daytime hypersomnolence, inattention and cognitive deficits, is characterized by recurrent arousals from sleep, related to specific stimuli (respiratory or motor events) or without any identifiable causes.

Arousals from sleep, spontaneous or induced, are associated with substantial autonomic activation, characterized by transient increase in blood pressure, heart rate and ventilation [18]. Cardiovascular modifications are present either in arousals with cortical involvement; "cortical arousals", characterized by the appearance of alpha or low voltage fast EEG activity, either in "subcortical arousals" coupled with sequences of delta waves or K-complexes without any EEG desynchronization [19, 20]. Consequently different techniques have been applied to detect changes in cardiovascular parameters as markers of arousals. Due to the non-stationary pattern of the data WT has been proposed.

Spectral analysis of HRV calculated with WT from nocturnal polygraphic recordings, provides indirect measures of sleep fragmentation. In particular, an increase in the LF/HF ratio was related to the arousal index and was observed despite the nature of the arousal [21]. Likewise, WT was applied to quantify the recurrence of vagal stimulation and sympathetic discharge induced by sleep apnoeas during full night ECG recording of patients examined for possible sleep-related breathing disorders. The alteration in the power coefficient of specific wavelet variables was highly predictive of OSAS, so that time frequency domain analysis of HRV using WT could be considered an efficient diagnostic marker of this breathing disorder [22].

Lastly, WT has allowed investigation of the temporal relationship between autonomic modifications and cerebral and muscular activity related to periodic limb movements of sleep (PLMS), stereotyped, involuntary and repetitive limb movements mostly occurring in NREM sleep and less frequently during REM sleep.

Using WT for the spectral analysis of EEG activity and HRV, an early sympathetic activation was found to precede EEG changes and movement onset in PLMS, suggesting a primary role of the sympathetic nervous system in the generation of PLMS [23]. Other studies applying WT to EEG spectral analysis and evaluating variation in HR expressed by RRi showed a similar sequence of changes consisting in a significant increase in HR and EEG delta power a few seconds before the onset of PLMS. According to these data, cardiac activation, together with delta EEG activity increase, is probably a preparatory condition triggering PLMS, and not a consequence of the movement [24, 25].

Despite methodological differences in the studies analyzed (different MFs, sampling rates,...), the WT approach appeared a powerful tool to detect a sudden shift in autonomic nervous system balance associated with transient phenomena, and could therefore be

applied to investigate cardiovascular changes accompanying the epileptic motor phenomena of sleep.

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1.3. NOCTURNAL FRONTAL LOBE EPILEPSY

1.3.1. Clinical and seizure characteristics

Nocturnal frontal lobe epilepsy (NFLE) is a distinct syndrome of partial epilepsy whose clinical features comprise a spectrum of paroxysmal motor manifestations of variable duration and complexity, arising mainly from sleep.

Three main semeiological patterns were observed in a large series studied by video-polysomnographic recordings [1]:

1. minor motor events (<20 seconds) characterized by brief, sudden and recurrent arousals from sleep associated with frightened or surprised expression and stereotyped movements of the head, trunk and limbs (paroxysmal arousals-PA);
2. major attacks (20 s-2 minutes), originally named nocturnal paroxysmal dystonia (NPD) [2], that include asymmetric tonic seizures (ATS) and more complex motor episodes with violent, uncoordinated and repetitive movements of the limbs and trunk and vocalizations (hyperkinetic seizures-HS);
3. prolonged episodes (1-3 minutes) consisting of a stereotypic paroxysmal ambulatory behaviour (epileptic nocturnal wandering-ENW).

These three different manifestations coexisted in the same patient, and the beginning of the ictal motor pattern was usually stereotyped. Investigations with intracerebral recording techniques during presurgical evaluation in patients with drug-resistant NFLE seizures demonstrated that the increasing complexity of the motor behaviors from minor events to prolonged seizures reflects different duration, amplitude and spread of the epileptic discharge [3].

The frequency of NFLE seizures was reported to be usually high (mean of 20 seizures monthly and 3 attacks nightly) [1]. However, in the large series studied by Provini [1] most

patients (72%) were not aware of their nocturnal attacks, which were described by their relatives. Nevertheless patients often complained of nocturnal sleep discontinuity due to frequent arousals and daytime sleepiness-related symptoms.

Seizures could occur during any time of the night, mainly from stages 1-2 NREM sleep and occasionally during REM sleep. Electroencephalographic (EEG) activity during the seizures failed to disclose epileptic activity in almost 50% of patients. Diffuse or focal flattening of background activity and appearance of rhythmic theta or delta activity were the prominent rhythms observed in the majority of patients presenting abnormalities, but only 10% showed spike and wave activity, while another 10% showed focal fast activity. Interictal EEG activity was frequently normal, with clear-cut epileptiform discharges observed in only 33% of individuals in wakefulness and 45% in sleep [1].

Neuroradiological investigations by means of brain CT or MR scans were poorly informative and most cases investigated with non invasive techniques resulted cryptogenic [1, 4].

NFLE has been described to occur sporadically or as an inherited form associated with mutations in the genes encoding the α4 (CHRNA4), α2 (CHRNA2) and β2 (CHRNB2) subunits of the neuronal nicotinic acetylcholine receptors (autosomal dominant nocturnal frontal lobe epilepsy -ADNFLE-) [5]. Clinically, sporadic and familial cases of nocturnal frontal lobe epilepsy had similar clinical presentation. A positive family history for epilepsy was recognized in 25% of patients and the occurrence of one or more parasomnias in at least one first degree relative was common in patients with NFLE [1].

The prognosis of NFLE is partially benign, as most patients have responded favorably to antiepileptic drugs, particularly carbamazepine [1]. In patients with drug-resistant disabling seizures resective surgery of the epileptogenic focus led to effective control of seizures and epilepsy-related sleep disturbances [4]. In these patients non invasive anatomical and

electrophysiological presurgical evaluations often failed to identify the region of seizure onset so that investigations with intracerebral recording techniques were required [4].

1.3.2. NFLE, NREM parasomnias and the unifying role of arousal

As previously explained, EEG findings and neuroimaging investigations are often poorly informative in NFLE patients leading to problems of differential diagnosis between epileptic and non-epileptic paroxysmal sleep-related phenomena presenting with similar motor features [6]. In particular, NFLE seizures have often been misdiagnosed as "disorders of arousal" (DA) which are parasomnias arising from NREM sleep including "confusional arousals", "sleepwalking" and "sleep terrors" [7]. Arousal parasomnias are the expression of sleep wake state dissociation in which wakefulness and NREM sleep seem to coexist.

Polysomnographic recording of the whole night with continuous audiovisual monitoring and careful history-taking are necessary to establish a correct diagnosis [6]. According to clinical history, the following characteristics could be useful for the differential diagnosis: age at seizure onset (3-8 years for DA, any age for NFLE), seizure frequency (low for DA, even several per night for NFLE), influence of triggering factors (ascertain for DA, absent for NFLE) and disease evolution (spontaneous disappearance of nocturnal episodes throughout life in DA). Video-polysomnography documented that NREM parasomnia episodes arise from NREM sleep stages 3-4 typically in the first part of the night, do not generally occur with a stereotypical motor pattern and abnormal dystonic or diskinetic features are absent . Instead, the recording of several motor events, any time during the night, usually with brief duration (seconds) and stereotypic clinical features are indicative of NFLE seizures [6]. However, experts did not always reach a consensus on the diagnosis especially when classifying brief motor phenomena as PA or non epileptic arousals [8].

The coexistence of parasomnic attacks in patients with NFLE or in their relatives [1, 9] is another confounding feature.

The difficulties encountered in the differential diagnosis between these paroxysmal motor behaviours during sleep, could otherwise reflect a common pathophysiological mechanism, originating from the pathways controlling the occurrence of physiological arousal during sleep [10].

Arousal responses during sleep are characterized by a continuous spectrum of EEG changes, ranging from high voltage slow rhythms (K-complexes and delta burst) to low amplitude fast rhythms, associated with various degrees of autonomic and somatomotor changes [11-13].

A sequence of EEG arousals recurring at brief intervals during sleep have been associated with a condition of sleep instability and proved to be favourable triggers in several sleep-related physiological [14] and pathological motor phenomena [15]. During these periods of unstable sleep EEG arousals (vertex sharp waves, K-complexes, delta busts) tend to occur in repetitive sequences lasting 8-15 s (phase A) separated by intervals of 15-20 s (phase B) of transient reappearance of background EEG activity [16]. This endogenous biphasic rhythm pattern is know as a cyclic alternating pattern (CAP) where phase A of CAP reflects a condition of transient activation and acts as a gate facilitating the occurrence of motor events.

Both major and minor motor episodes of NFLE have been associated with the slow component of phase A (K-complexes and delta burst) [15] and sound-induced K-complex arousals were demonstrated to trigger seizures in a patient with autosomal dominant nocturnal frontal lobe epilepsy [17]. Likewise, bursts of high-amplitude repetitive and monomorphic slow delta waves are usually observed before onset of NREM parasomnias [18].

Taken together, these findings suggest that arousal during sleep could represent a facilitating condition for NFLE and NREM parasomnias. It has been hypothesized that motor behaviours common to parasomnic and epileptic manifestations, derive from the release of central pattern generators (CPGs), neuronal circuitry, mainly located in the meso-diencephalic-pontine regions and the spinal cord, capable of generating stereotyped motor patterns and whose activity is modulated by the cerebral cortex [19]. Increased arousal instability, intrinsically due to a sleep-related dysfunction, can lead to temporary loss of cortical control over CPGs and therefore facilitate the emergence of stereotyped inborn fixed action patterns. Otherwise epileptic discharge recurrence in NFLE could be directly responsible for increased arousal fluctuation and sleep instability and hence facilitate activation of the same CPGs [20].

1.3.3. NFLE seizures and cardiovascular symptoms

Autonomic symptoms during NFLE seizures (tachycardia, sustained tachypnoea and irregular respiratory rhythm) have been observed in most patients [1], but the extent of these modifications and their relationship with seizure onset has not been described.

Recordings with intracerebral electrodes [3, 21] demonstrated that during NFLE seizures the epileptic discharge could arise from regions of the CAN devoted to cardiovascular modulation like the cingulate gyrus and insular cortex. In addition, SPECT studies showed increased blood flow in the right anterior cingulate gyrus and in the cerebellum during an episode of PA [22] and bilateral hyperperfusion of the anterior cingulate gyrus in a patient with NPD [23].

These data suggested that cardiovascular manifestations during NFLE are probably due to the direct effect of epileptic discharges. For this reason, analysing how cardiovascular

changes develop during seizures could provide useful information in the differential diagnosis with other non epileptic motor phenomenon occurring during sleep.

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1.4. CARDIOVASCULAR CHANGES IN EPILEPSY

1.4.1. Ictal cardiovascular changes

Cardiovascular manifestations frequently occur during epileptic seizures [1-3] and have received particular attention due to their possible role in the pathogenesis of sudden unexplained death [4-5]. Investigations of ictal HR changes could also provide information on the localization or lateralization of the seizure onset zone.

The most frequently reported cardiovascular change associated with seizures is ictal tachycardia (IT), whereas ictal bradycardia (IB) is much less frequently observed.

1.4.1.1. Ictal tachycardia

Ictal sinus tachycardia, when investigated, was observed in more than 85% of seizures in different studies [6-9]. Heart rate has been reported to increase up to 120 beats per minutes (bpm) in 67% [6] to 76% of seizures [7], with possible peak frequency exceeding 200 bpm, without major clinical haemodynamic consequences.

Heart rate changes were observed to precede, follow or coincide with seizure onset [9, 11-12]. The temporal relation between the onset of ictal EEG discharges and changes in ictal HR was evaluated in detail to disprove the hypothesis that HR changes might be solely a consequence of ictal motor activity. In 145 complex partial seizures, IT, defined as ictal HR increase >1 standard deviation of the mean preictal HR of all patients, was found to precede the ictal discharge, evaluated through scalp recording, in more than 75% of seizures by 0.7–49.3 s [9]. In another study the ictal HR increase preceded the onset of the EEG discharge by 5 seconds in 98% of seizures of medial temporal lobe origin, while it coincided with the ictal onset discharge in 95% of seizures arising from the lateral temporal regions [12].

According to the cerebral region of seizure onset, IT was observed more frequently in seizures arising from the temporal lobe, especially from mesial structures, compared with seizures of extratemporal lobe origin [7, 9, 11]. Moreover, when IT precedes EEG seizure onset the time lag between the two events appeared longer in seizures of temporal lobe origin compared to extratemporal seizures [9].

Two distinct patterns of IT development have been recognized: a continuous steady increase in HR, significantly related to temporal lobe seizures, and an abrupt increase in HR within a few RR intervals followed by continuous and steady increase, which occurred more often in seizures of extratemporal origin [9]. In addition, patterns of HR changes were relatively stereotyped across different seizures within a given patient, suggesting that they might be related to specific patterns of seizure spread [9, 13].

There is current no consensus on the hypothesis of lateralized hemispheric influences in determining the degree of ictal HR increase [7-12]. Some claim the prevalence and magnitude of IT depend principally on the generalization of EEG seizure discharge [6, 8, 14] and the volume of cerebral structures recruited during the seizure [15].

1.4.1.2. Ictal bradycardia

Ictal bradycardia has been reported in <6% of complex partial seizures [7, 13-14], and asystolic episodes associated with IB seemed to occur even less frequently (0.3-0.4%) [16-17]. However, the true incidence of this rare but potentially life-threatening condition is probably underestimated. Diagnosis of IB is established after documentation of bradycardia/asystole clearly determined by a documented concomitant ictal EEG discharge. Patients with bradyarrhythmias are usually admitted to coronary care units, where EEG investigations are not routinely performed, leading to possible misdiagnosis.

Moreover IB and/or ictal asystole (IA) do not necessarily occur in all seizures, and a limited number of seizures can be recorded during inpatient monitoring [18].

Unlike IT, IB usually starts 10-30 s after EEG seizure onset [16, 18] and could progress to IA which usually lasts 10-30 s. Both IT and IB could be preceded or followed by opposite HR changes. A pattern of HR changes consisting of tachycardia at the onset of the seizure evolving into progressive bradycardia leading to asystole has been described in seizures associated with IB. After IA a reversed pattern was observed characterized by spontaneous sinus bradycardia followed by brief tachycardia before HR returned to baseline values [17].

IB is seen primarily during seizures arising or involving the temporal lobe [18-19], but cases of IB related to orbitofrontal lobe seizures have also been reported [20].

Lastly, IB does not appear to be a lateralizing sign in the localization of seizure onset occurring in association with discharges arising either from the left or right hemispheres [18] or after bilateral hemispheric spread of seizure activity [19]. Moreover the lateralization hypothesis cannot explain the combined pattern of HR decrease and increase observed during IB. These data and similar results in cases of IT support the hypothesis of the influence of the seizure onset zone in determining the prevalence, direction and degree of ictal HR changes. On the contrary, cerebral cortex lateralization in parasympathetic and sympathetic cardiac control has not been confirmed.

1.4.2. Ictal and interictal cardiovascular changes and SUDEP

Epileptic patients have an increased risk of sudden death with an incidence ranging from 0·09 per 1000 patient-years in newly diagnosed patients to 9 per 1000 patient-years in candidates for epilepsy surgery. SUDEP is defined as the sudden, unexpected, witnessed or unwitnessed, non-traumatic, and nondrowning death of patients with epilepsy with or

without evidence of a seizure, excluding documented status epilepticus, and in whom post-mortem examination does not reveal a structural or toxicological cause for death.

Although no definitive evidence has emerged from animal models or clinical data, ictal cardiac changes might play a role in the pathophysiology of SUDEP [5]. Although IT does not have major clinical haemodynamic consequences, it can be associated with rare but life-threatening cardiac anomalies like atrial fibrillation [14, 21], or ST depression and T-wave inversion [8, 22, 23]. Likewise, IA that seemed to be a self-limiting phenomenon, may last long enough to become potentially dangerous directly [24] or by inducing atonia and fall [25]. Pacemaker implantation in patients with IB, particularly those with drug-resistant seizures, can prevent these adverse effects.

Recognizing cardiac ictal arrhythmias is also useful to choose the correct antiepileptic therapy as some antiepileptic drugs have cardiovascular side-effects and contribute to SUDEP. Carbamazepine has been reported to exert a negative chronotropic and dromotropic effects [26], particularly in patients with cardiac electrophysiological abnormalities [27]. Carbamazepine was also present in chronic therapy in some series of epileptic patients presenting with SUDEP [28]. Phenytoin was shown to act by centrally depressing hyperactivity in cardiac sympathetic nerves and abolishing arrhythmias [29], and hence may be beneficial in patients with IT, but should be avoided in patients with IB due to its cardioinhibitory action [18].

Interictal cardiovascular autonomic dysregulation has also been described in epileptic patients and could contribute to SUDEP. Several studies investigating HRV in steady state conditions and in response to standard autonomic tests found interictal changes, including reduced overall HRV, decreased sympathetic or parasympathetic activity or a combined reduction of both, or low parasympathetic tone associated with high sympathetic tone [3].

Impaired baroreflex functions in epileptic patients [30] and autonomic changes during sleep have also been documented [31].

Mechanisms leading to the shift of the sympathoparasympathetic balance toward the dominance of one autonomic system over the other are not yet clearly understood, but are likely to result from progressive alterations induced in autonomic centres by repetitive seizure discharges [3].

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2. OBJECTIVES

The primary aim of the study was to analyze HR changes, expressed as RRi variations, occurring during NFLE seizures. We were particularly interested in the development of RRi changes with respect to the motor onset of the seizure according to the hypothesis that cardiovascular manifestations are not only due to ictal motor behaviors, but also reflect epileptic discharges involving regions of the CAN. As scalp EEG activity preceding clinical seizure onset tends to be poorly informative in NFLE seizures, we evaluated whether RRi variations could be considered an early clinical sign and hence a reliable diagnostic marker of seizure occurrence.

Secondarily, we applied the wavelet transform technique using time and frequency domain analysis of heart rate variability to determine the sudden and transient changes in sympathovagal balance occurring during seizures, again specifically with regard to the clinical motor seizure onset. In doing this, we proposed to search for centrally mediated indicators of autonomic activation preceding and possibly resulting in seizures.

Lastly, if a unique pattern of cardiovascular changes proved to be associated with NFLE seizures, we planned to assess its contribution in the differential diagnosis with other motor phenomena occurring during sleep.

3. METHODS

3.1. PATIENTS and RECORDINGS

3.1.1. Patients

We retrospectively reviewed 18 consecutive patients who underwent a whole night digitally recorded video-polysomnography (VPSG) for NFLE in our sleep centre from 2000 to 2006. Only two out of 18 patients followed the inclusion criteria and were considered for the study.

From 2007 to 2009 we evaluated for the study another nine consecutive patients diagnosed with NFLE, two were excluded after VPSG.

The nine patients finally included met the following criteria:

1. Diagnosis of NFLE formulated in the presence of an evocative history and after VPSG recording of at least two episodes with a stereotypic motor pattern suggestive of NFLE seizures;
2. Absence of an active cardiovascular disease or any other disorder that might affect the autonomic nervous system;
3. Absence of other neurological or mental disorders;
4. Exclusion of a sleep-related breathing disorder with VPSG;
5. Absence, at the time of VPSG, of medications that could modify HR except for anti-epileptic drugs that if present were reduced or withdrawn two weeks before the VPSG;
6. Recording of at least two seizures during VPSG meeting the criteria explained below.

For all patients a full neurological examination, routine EEG recording during wakefulness, and neuroradiological evaluation (Brain MRI) were performed.

3.1.2. Recordings

Whole night VPSG (from 11 pm to 7 am) included standard bipolar scalp EEG (according to the International 10-20 System), surface right and left electro-oculogram (EOG), electromyogram (EMG) of mentalis, electrocardiogram (ECG) (from a standard D2 lead), and thoracic and abdominal respirograms (strain-gauge). EMG of at least one limb muscle, selected according to the anamnestic description of the clinical onset of the seizure, was also performed.

Continuous audiovisual acquisition was available for seven patients while for the other two video recordings were limited to the seizure period.

Data were recorded with two digital acquisition systems in seven patients: *Connex EEG Sleep-XLTEK Software 5.4* (sampling rate 256 Hz; 4 patients) and *Nihon Kohden EEG-1200* (sampling rate 500 Hz; 3 patients). For two patients data were acquired on a *Grass polygraph* with paper speed of 10 mm/s (30 s epoch) and synchronized video-recording and were computerized and stored with Neuroscan Acquisition System P/N 1098, SCAN 4.2 (sampling rate: 256 Hz).

3.1.3. Criteria for seizure selection

VPSG were revised independently by three examiners (P.T.; F.P.; F.B.) and events with features of NFLE seizures were selected and included in the study only if a consensus was reached. In addition, we chose for HR analysis only seizures occurring at least three minutes after the end of a previous seizure and 90 seconds from the end of another

spontaneous or evoked arousal or another motor phenomenon. This criterion was adopted to avoid the confounding persisting HR changes due to seizures or other phenomena preceding the seizure under analysis. Seizures with loss of ECG signal due to movement artefacts were excluded.

3.2. ANALYSIS

3.2.1. Seizure analysis

For each seizure clinical classification, sleep stage of occurrence and duration were assessed. Seizures were classified according to two main semeiological patterns: paroxysmal arousal (PA), minor motor events characterized by brief, sudden and recurrent arousals from sleep associated with frightened or surprised expression and stereotyped movements of the head, trunk and limbs, and nocturnal paroxysmal dystonia (NPD) that includes asymmetric bilateral tonic seizures (ATS) and more complex motor episodes with violent, uncoordinated, and repetitive movements of the limbs and the trunk, and vocalizations (HS) [1]. Seizures with features of episodic nocturnal wandering (ENW), consisting of prolonged episodes with a stereotypic paroxysmal ambulatory behaviour, were excluded due to the unavoidable loss of the ECG signal.

Sleep stages were visually scored on 30 s epochs, according to the American Academy of Sleep Medicine criteria, as light NREM sleep (stages 1 and 2), deep NREM sleep (stages 3), and REM sleep [2].

Seizure onset was identified with the clinical onset of the seizure, corresponding to the first significant change in EMG activity or the first movement observed in the video recording. Seizure period corresponded to the development of clinical epileptic manifestations and was visually defined from the video recording.

3.2.2. Heart rate analysis

Heart rate was evaluated by measuring the interval between two consecutive R-waves of QRS complexes (RRi). RRi series were digitally identified and automatically calculated for

a period of 20 minutes, including the seizures, by means of Vision Analyser software (version 1.05-Brain Products). Visual control of RRi series allowed erroneous R waves and missed detection to be corrected. Ectopic beats were deleted from the resulting RRi series and replaced by a virtual beat by interpolating adjacent R waves as recommended [3].

3.2.3. Spectral analysis of heart rate variability

Spectral analysis of HRV was performed to assess seizure-related changes in autonomic activity. The power spectrum of HRV comprises high-frequency components (HF: 0.15-0.40 Hz), reflecting parasympathetic outflow and breathing activity, low frequency components (LF: 0.04-0.15 Hz), mediated mostly by sympathetic activity and very low frequency components (VLF: 0÷0.04 Hz) whose meaning is controversial. The LF/HF ratio is widely used to indicate the balance between sympathetic and parasympathetic outflows.

A discrete wavelet transform (WT) was preferred to the classical Fourier transform to describe the temporal evolution of the frequency spectrum contained in the ECG signal. As previously explained, WT does not assume stationarity of the analyzed signal and may detect transient and rapid changes in HRV.

Analysis was conducted off-line over a period of 20 consecutive minutes for each seizure using Mathematica® 7.0. Period analysis length of at least 20 minutes was demonstrated in our preliminary evaluations to reproduce the same power spectrum of HRV obtained with the whole night analysis.

RRi series were firstly resampled at 10 Hz using cubic spline interpolation following the heuristic rationale of resampling at approximately ten times the band of the fastest regulatory mechanism of interest (which is assumed to be active beat-to-beat) [4].

A multiresolution analysis was then performed using the Daubechies-16 form as mother function. WT signal decomposition requires an adequately regular and localized basal function called the ‘‘mother function (MF).’’ In our study we chose the Daubechies-16 form to guarantee a steep fall-off at the boundaries of the mother wavelet spectrum and a close match between the full width at half maximum boundaries of dilated wavelet spectra and the HF and LF band transition frequencies.

A family of basis functions which are scaled versions of the MF was then built by dilation and translocation of the MF. The similarity between the signal and these basis functions were estimated through coefficients computed by convolving the original signal with the basis functions in the time domain.

The squared level specific amplitude coefficients were summed across appropriate decomposition levels to compute total band powers in bands of interest (VLF: 0.00976551 - 0.039062, LF: 0.039062 - 0.156248, HF: 0.156248 - 0.624992) (Figure 2).

The first levels of transformation (1-17) correspond to a wavelet analysis conducted with a small value of the dilation factor, thus representing high-frequency variations in the signal. On the contrary, the last levels correspond to a wavelet analysis conducted with a large value of the dilation factor, and representing low-frequency (17-74) and very low-frequency (74-255) variations in the signal. At any level, the larger the coefficients were, the greater the correspondence between the original signal and the analyzing wavelet.

Band transitions/edges were estimated as the points of half-maximum spectral power amplitudes of the level-specific MF dilations. This approach was preferred to the traditional one of quoting the central frequency of the dilated wavelets to take in to account the individual shape of the mother wavelet spectrum, hence better estimating the bands related to the coefficients resulting from wavelet transform application.

Band specific powers were then filtered using a linear recursive filter (exponential filtering with $\alpha = 0.1$) to reduce noise especially in the HF band.

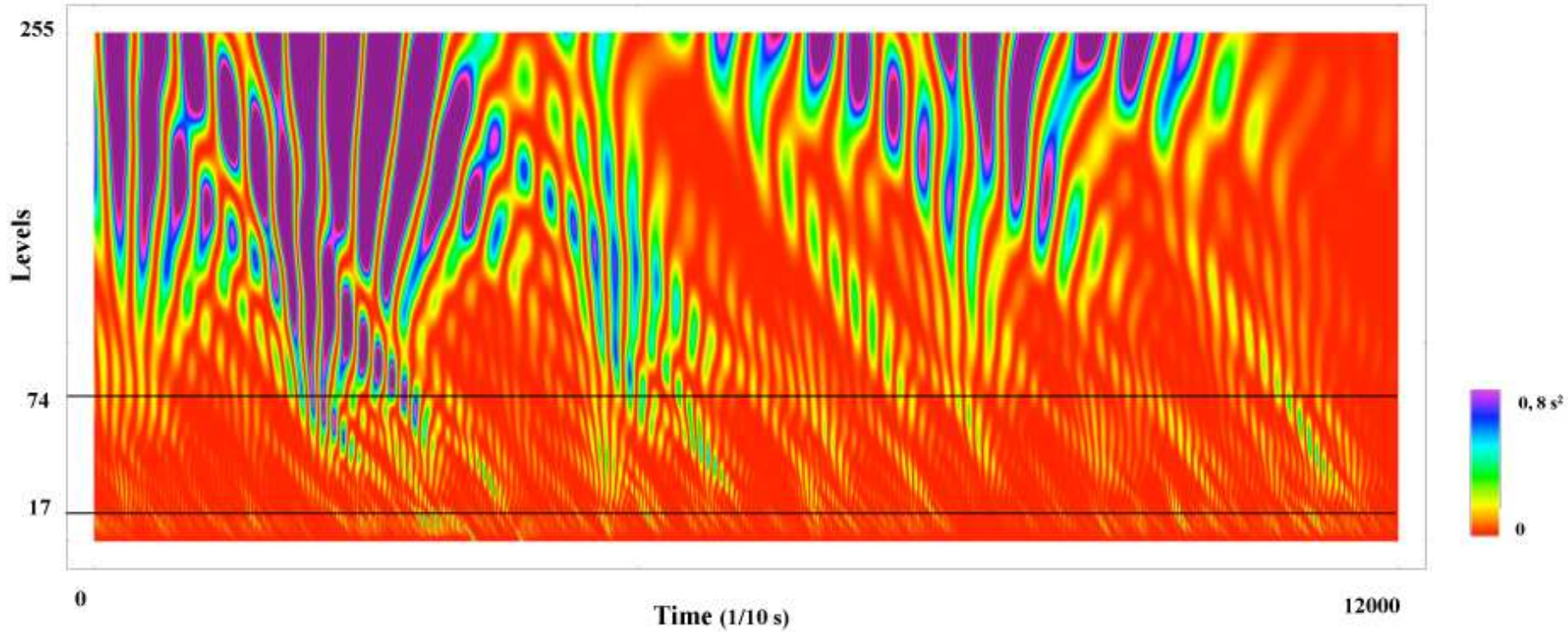

Figure 2. Wavelet analysis performed on the RRi series, resampled at 10 Hz, of 20 consecutive minutes selected from the whole night recording of patient 2. The figure showed the ability of wavelet analysis to detect sudden frequency variations along a time scale. Black horizontal lines delimit transformation levels representing different signal frequencies: HF:1-17; LF: 17-74; VLF: 74-255.

3.2.4. Statistical analysis

3.2.4.1. Selection of analysis periods

Before proceeding with statistical analysis we plotted the values obtained for each parameter of interest (RRi, LF and HF) (y-axis) versus time (x-axis) and found that most of these graphs followed a specific pattern for seizures of different patients.

We consequently defined three analysis periods:

1) Basal period (Basal): 30 seconds, at least 30 seconds before seizure onset during which no movements occurred and autonomic conditions, evaluated by the LF/HF ratio assessed with WT, were stationary;

2) Pre-seizure period (preSP): 10 seconds preceding seizure onset;

3) Seizure period (SP): corresponding to clinical motor manifestations visually defined from the video-recording.

3.2.4.2. Data and analysis

RRi values (s) and HF and LF absolute values (s2) were used for statistical analysis. We selected one value every ten out of the 10 Hz sequences of resampled data to have a time resolution of 1 second. Initially we performed a group analysis considering all patients together, followed by a single subject analysis.

Data are reported as means ± SD. Data non-normally distributed (LF and HF absolute values) were transformed using logarithmic transformation to resemble normal distribution.

For **group analysis** a general linear univariate model (factorial ANOVA) was applied to estimate changes in each parameter (RRi, LF and HF), during the three defined periods, assuming the parameter as a dependent variable and alternatively patient, seizure, sleep phase of occurrence and period as fixed factors (see results for details).

RRi values were considered covariates in the LF and HF statistical model to minimize the effects of RRi differences between patients.

Lastly, as LF correlated significantly with HF, we assumed HF as covariate in the LF model to reduce the effect of differences in the LF variable due to different HF values.

For **single subject analysis** a general linear univariate model (factorial ANOVA) was applied assuming again the parameter of interest as dependent variable (RRi, LF and HF) and period as fixed factor. Seizure was considered a fixed factor only when it was a significant predictor of the dependent variable.

All statistical analyses were performed with SPSS-PASW (Predictive Analytics Software) version 18 and significance was set at $p \leq 0.05$.

Data of individual subjects were also measured as normalized units representing the percentage of LF and HF component in relation to the total power except for the VLF component, and as LF/HF ratio. Even though they were not used in the statistical analysis, the normalized indices and the ratio were plotted when necessary to better represent the modulation exerted by the two branches of the autonomic nervous system.

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4. RESULTS

4.1. PATIENTS and SEIZURES

Nine patients were selected for the study. Patient 9 underwent a whole night video-polysomnographic (VPSG) recording and prolonged 24-hour video-polygraphic monitoring during which three seizures associated with ictal asystole were recorded, and described in detail below. We finally included in the group analysis eight patients (5 males, 3 females; mean age at VPSG: 24±9 years), whose clinical features are summarised in Table 1.

Age at seizure onset ranged from three to 14 years (mean 9±4 years). Four patients (1,3,7,8) had a positive family history for parasomnias, two patients (3,6) had a history of sleep enuresis and one (1) had febrile convulsions. Interictal wakefulness EEG and brain MRI were uninformative in most patients. Interictal sleep EEG showed clear epileptic abnormalities in three patients (1,2,6).

At the time of VPSG seizure frequency was high in all patients (Table 2). Six patients were under antiepileptic drugs (AEDs) and presented drug-resistant seizures. Antiepileptic drugs were reduced in five patients but withdrawn only in one to avoid the occurrence of secondarily generalized seizures.

The number of seizures analyzed varied between patients, ranging from two to eight (Table 2). All 41 seizures (11 PA; 30 NPD) arose from NREM sleep (mean duration PA: 8±5.6 s; mean duration of NPD except for Pt 6: 27±10.3 s). Seizures in Patient 6 lasted longer than three minutes comprising a first part with hyperkinetic automatism (mean duration: 106±21 s), and a second part (mean duration: 76±15 s) characterized by a clear EEG

epileptic discharge in the left centro-fronto-temporal regions associated with behavioural arrest, unresponsiveness and assumption of an asymmetric posturing of the right arm and leg (see single patient analysis for description).

Six patients showed ictal EEG abnormalities but only Patient 6 presented a clear epileptic ictal activity.

After VPSG Patient 8 underwent presurgical evaluation with deep implanted electrodes that identified the seizure onset zone in the right cingulate gyrus. He subsequently received a microsurgical resection of the epileptic zone (posteromesial frontal cortex and cingulate gyrus) with a seizure-free outcome.

Patient	Sex	Family history	Personal history	Age at seizure onset (years)	Frequency of nocturnal seizures at VPSG	Interictal EEG (wakefulness)	Interictal EEG (sleep)	Brain MRI
1	F	Parasomnias (sleep-walking/ sleep terrors)	FC	5	Several per night	Normal	B fronto-temporal spikes	Normal
2	F	-	-	14	Several per night	Centro-frontal spikes	B centro-frontal spikes	Normal
3	M	Parasomnias (sleep-walking/ sleep terrors)	Sleep enuresis	14	Several per night	Normal	Normal	Normal
4	M	Negative	-	14	1-3 episodes per night, 1-2 nights per week	Normal	Normal	Normal
5	M	Negative	-	3	3-4 per night	Normal	L temporal and R centro-frontal theta activity	Normal
6	M	Negative	Sleep enuresis	8	Several per night	Normal	R and L fronto-temporal spikes and sharp waves	Normal
7	F	Parasomnias (sleep-walking)	-	9	Several per night	Normal	Posterior vertex and R parietal theta activity	Normal
8	M	Parasomnias (sleep-walking)	-	6	Several per night	Normal	B fronto-temporal theta activity	Normal

Table 1: **F:** female; **M:** male; **FC:** febrile convulsions; **L:** left; **R:** right; **B:** bilateral.

Patient	Age at VPSG (years)	AEDs at VPSG (mg/die)	No. of nights analyzed	No. of seizures recorded	No. of seizures analyzed	Seizure features	Sleep phase of occurrence			Seizure duration Mean ± SD (s)	Ictal EEG
							NR1	NR2	NR3		
1	23	CBZ 800; TPM 50 **R:** CBZ 400; TPM 50	1	10	7	NPD (HS)	-	3	4	23 ± 2.24	Fast, low voltage diffuse activity
2	23	CBZ 1000; LTG 300 **R:** CBZ 600; LTG 200	1	4	4	NPD (ATS)	1	1	2	22 ± 3.37	Fast, low voltage diffuse activity
3	34	-	2	17	8	PAs	-	2	6	8 ± 6.51	Normal
4	41	-	1	4	3	PA	-	-	3	10±2	Normal
5	20	CBZ 1000; CLB 20 **R:** CBZ 800	2	3	2	NPD (HS)	-	2	-	34±10.6	L temporal flattening and rhythmic theta activity
6	19	CBZ 800 **Withdrawn**	1	8 NPD, Several PA	3	NPD (HS)	-	2	1	205±16	L fronto-centro- temporal rhythmic fast activity
7	23	CBZ 1000; CZP 1.5 **R:** CBZ 400	1	12 NPD, Several PA	7	NPD (ATS)	-	6	1	37±13.3	R centro-parietal theta rhythmic activity
8	12	CBZ 600; LTG 50 **R:** CBZ 400	1	11 NPD, Several PA	7	NPD (ATS)		2	5	20±4.7	B centro-fronto-parietal theta rhythmic activity

Table 2: VPSG: video-polysomnography; **AEDs:** antiepileptic drugs; **CBZ:** carbamazepine; **TPM:** topiramate; **LTG:** lamotrigine; **R:** reduced**; NPD:** nocturnal paroxysmal dystonia; **HS:** hyperkinetic seizures; **ATS:** asymmetric tonic seizures; **PA**: paroxysmal arousal; **L:** left; **R:** right; **B:** bilateral.

4.2. CARDIOVASCULAR PARAMETERS: GROUP ANALYSIS

4.2.1. Analysis of RRi

Mean RRi values ± SD during the three different periods for each patient are listed in Table 3. A general linear model was first applied assuming RRi as dependent variable and sex, patient, sleep phase of occurrence (NREM1, NREM2, and NREM3), seizure, and period as fixed factors. Propriety of the model, expressed by Fisher's F was 166, corresponding to a significance of p <0. 0001.

As only one seizure occurred in phase NREM1, this seizure was excluded from the analysis. We subsequently simplified the analysis model excluding sex and seizure as fixed factors (F =412; p<0. 0001).

As sleep phase was not a significant predictor of RRi (p=0.249) it was also excluded from the model. The final model assumed RRi as dependent variable and patient and period as fixed factors (F= 708.8; p <0.0001). The analysis indicated a significant period effect on the RRi variable: compared to basal values, RRi remained unchanged during the preSP, whereas it significantly decreased during the SP (p<0.001). Results were consistent in the three models described. Estimated marginal means for overall RRi values obtained with the final model of analysis (Basal: 1.036 s; preSP: 1.011 s; SP: 0.67 s) are reported in Figure 3.

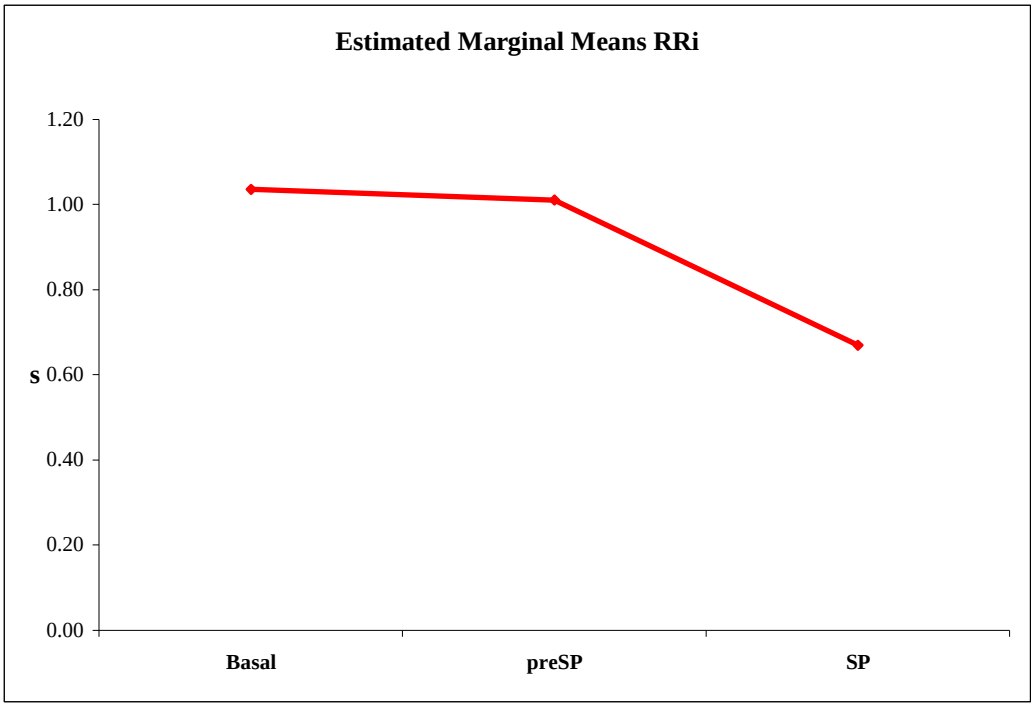

Estimated Marginal Means RRi
1.20
1.00
0.80
s 0.60
0.40
0.20
0.00
Basal
preSP
SP

Figure 3.

Patient	Period	Mean RRi (s)	SD
1	Basal	0.8682	0.0551
	preSP	0.8556	0.0543
	SP	0.5314	0.0861
2	Basal	0.7990	0.0367
	preSP	0.7887	0.0383
	SP	0.5896	0.0738
3	Basal	1.0475	0.0558
	preSP	1.0357	0.0558
	SP	0.6995	0.1811
4	Basal	1.0902	0.0720
	preSP	1.1179	0.0877
	SP	0.8096	0.0965
5	Basal	1.5287	0.0985
	preSP	1.4286	0.0773
	SP	0.9975	0.1546
6	Basal	1.0642	0.1270
	preSP	1.0102	0.1900
	SP	0.6157	0.1070
7	Basal	0.9974	0.1071
	preSP	0.9858	0.1150
	SP	0.4261	0.1284
8	Basal	0.8770	0.1033
	preSP	0.8557	0.1144
	SP	0.6859	0.1271

Table 3. Mean RR interval (RRi) values ± SD for each patient during the three defined periods. **Basal:** basal period; **preSP**: pre seizure period; **SP**: seizure period.

4.2.2. Analysis of HF

Mean HF absolute values ± SD during the three different periods for each patient are listed in Table 4. A general linear model was first applied assuming HF as dependent variable, patient and period as fixed factors ($F=149.3$; $p<0.0001$). We also used a model with HF as dependent variable, RRi as covariate, and period as fixed factor ($F=516$; $p<0.0001$). This model was chosen due to the increased F. Compared to basal values, a significant decrease of HF was observed during the SP ($p<0.0001$), whereas HF did not differ during the preSP. Results were the same in the first model evaluating patient and period as fixed factors. Estimated marginal means for Log HF during the three defined periods obtained from the group analysis are reported in figure 4.

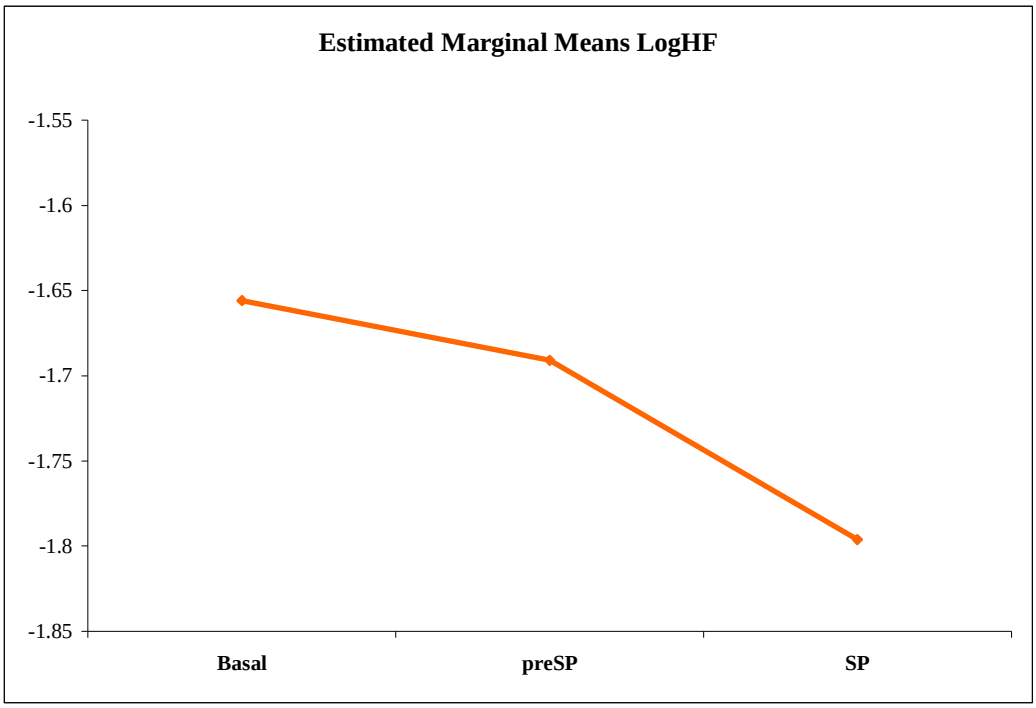


Figure 4.

Patient	Period	Mean HF (s^2)	SD
1	Basal	0.0323	0.0174
	preSP	0.0214	0.0109
	SP	0.0086	0.0123
2	Basal	0.0080	0.0046
	preSP	0.0048	0.0037
	SP	0.0076	0.0093
3	Basal	0.0102	0.0057
	preSP	0.0114	0.0058
	SP	0.0110	0.0116
4	Basal	0.0471	0.0236
	preSP	0.0365	0.0175
	SP	0.0205	0.0143
5	Basal	0.1062	0.1113
	preSP	0.1145	0.0603
	SP	0.0671	0.0627
6	Basal	0.2431	0.1190
	preSP	0.3411	0.2513
	SP	0.0578	0.1171
7	Basal	0.2247	0.1483
	preSP	0.2226	0.1593
	SP	0.0204	0.0442
8	Basal	0.2360	0.1397
	preSP	0.1682	0.0983
	SP	0.0760	0.0596

Table 4. Mean HF absolute values ± SD for each patient during the three defined periods.

Basal: basal period; **preSP**: pre seizure period; **SP**: seizure period.

4.2.3. Analysis of LF

Mean LF absolute values during the three different periods for each patient are reported in Table 5. A general linear model was applied assuming LF as dependent variable, period as fixed factor, and RRi and HF as covariates ($F=481.6$; $p<0.0001$). The analysis indicated a significant period effect on the LF variable. Compared to basal values, a significant increase in LF was observed during both the preSP ($p<0.0001$) and the SP ($p<0.0001$). The same results were obtained with a model ($F=151.4$; $p<0.0001$) considering LF as dependent variable, patients and period as fixed factors, and HF as covariate. Estimated marginal means for Log LF, during the three defined periods, obtained from the first model of analysis are reported in figure 5.

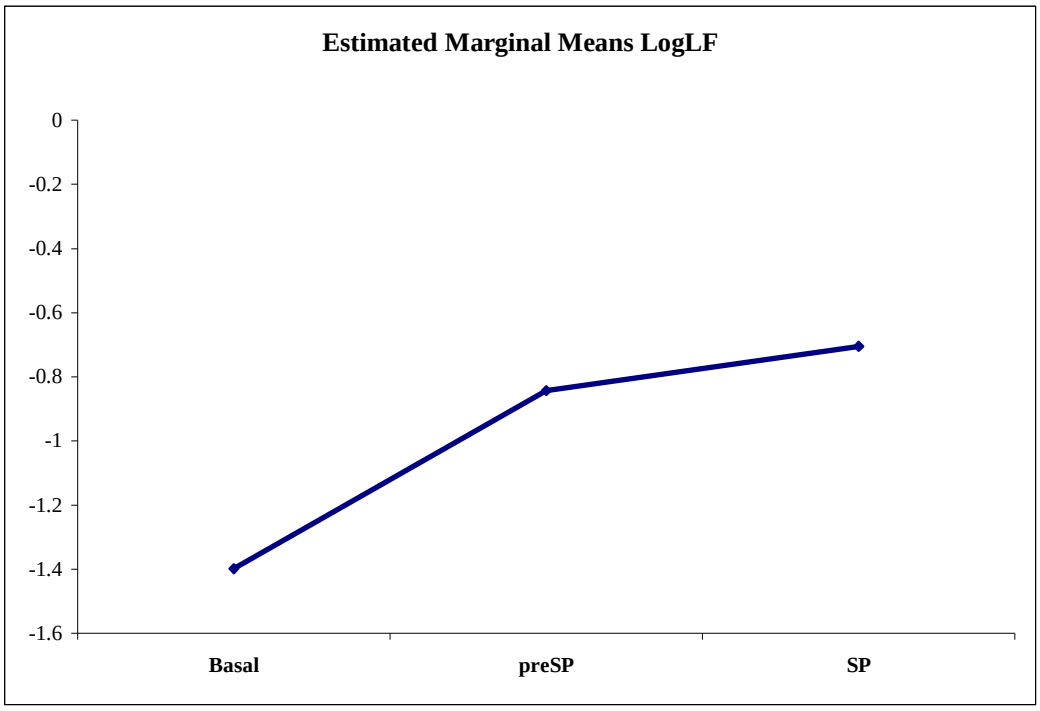


Figure 5.

Patient	Period	Mean LF (s^2)	SD
1	Basal	0.0632	0.0620
	preSP	0.2126	0.1506
	SP	0.1696	0.1479
2	Basal	0.0107	0.0064
	preSP	0.0564	0.0330
	SP	0.1791	0.1281
3	Basal	0.0352	0.0248
	preSP	0.1840	0.0947
	SP	0.2752	0.1322
4	Basal	0.0264	0.0207
	preSP	0.2158	0.1173
	SP	0.2012	0.1354
5	Basal	0.0905	0.0781
	preSP	0.4829	0.1861
	SP	0.7587	0.5536
6	Basal	0.2743	0.1876
	preSP	0.6398	0.4752
	SP	0.1843	0.2333
7	Basal	0.1691	0.1351
	preSP	0.3196	0.2377
	SP	0.1724	0.2409
8	Basal	0.1881	0.1248
	preSP	0.3905	0.3149
	SP	0.4806	0.3559

Table 5. Mean LF absolute values ± SD for each patient during the three defined periods. **Basal:** basal period; **preSP**: pre seizure period; **SP**: seizure period.

4.3. CARDIOVASCULAR PARAMETERS: SINGLE SUBJECT ANALYSIS

A general linear model was applied for the data of each patient assuming the parameter of interest as dependent variable (RRi, LF, and HF) and period as fixed factor. Seizure was included as fixed factor only if a significant difference in the values of the dependent variable between seizures was detected. Statistical analysis was performed only when more than three seizures were available. Before proceeding with the analysis we plotted the values obtained for each parameter of interest (RRi, LF, and HF) for the different seizures of each patient. Statistical analysis was performed only if a similar pattern of response for each parameter was visually observed in the majority of seizures of each patient. The characteristics of the model selected and results of the analysis for the parameters investigated are reported below for each patient.

4.3.1. Patient 1

Mean RRi, HF and LF values of the seven seizures analyzed are plotted in Figure 6. Single subject analysis for this patient were in accordance with the results of the group analysis for all the parameters evaluated (Table 6). HF values also tended to decrease in the PreSP but a significant difference was not reached.

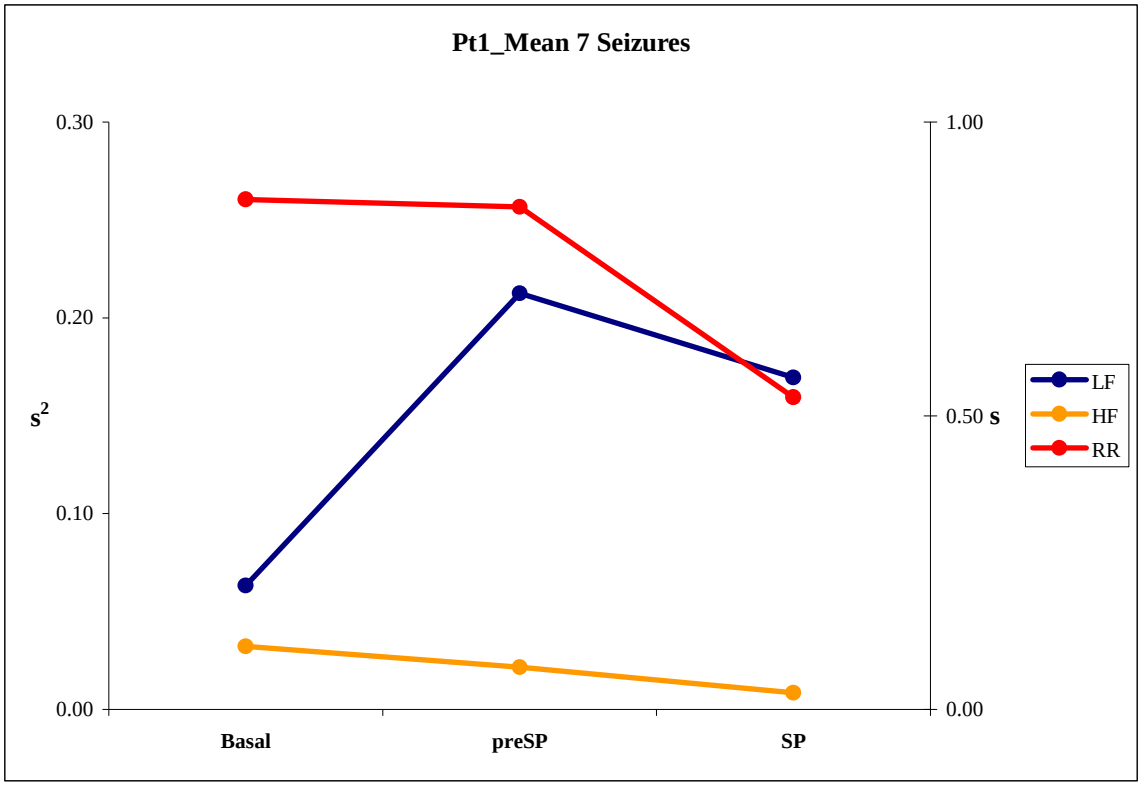


Figure 6.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Seizure Period	F=162.2 p<0.0001	B=-0.036 p=0.104	yes	B=-0.359 p<0.0001	yes
HF	Seizure Period	F=35.5 p<0.0001	B=-0.163 p=0.220	yes	B=-1.028 p<0.0001	yes
LF	Seizure Period	F=27.7 p<0.0001	B=0.716 p<0.0001	yes	B=0.569 p<0.0001	yes

Table 6. Single subject analysis of patient 1. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

4.3.2. Patient 2

Mean RRi, HF and LF values of the four seizures analyzed are plotted in Figure 7. Single subject analysis for this patient found an accordance with the results of the group analysis for RRi and LF values (Table 7). The pattern of HF changes differed in the four seizures of this patient (Figure 8) due to the different values of the HF in the basal period and to the different seizure duration. Absolutes HF values (Figure 9) and normalized values of LF and HF (Figure10 a, b) for seizures 1 and 3 were plotted to better explain changes in sympathovagal balance during the seizures. Compared to Basal, HF remain unchanged (S1) or decreased (S2) in the first part of the SP, whereas in the second part of the SP an increase in HF values contributed to the mean of HF values only in the shortest seizures (Figure 9). Changes in sympathovagal balance were the same during the two seizures (Figure 10).

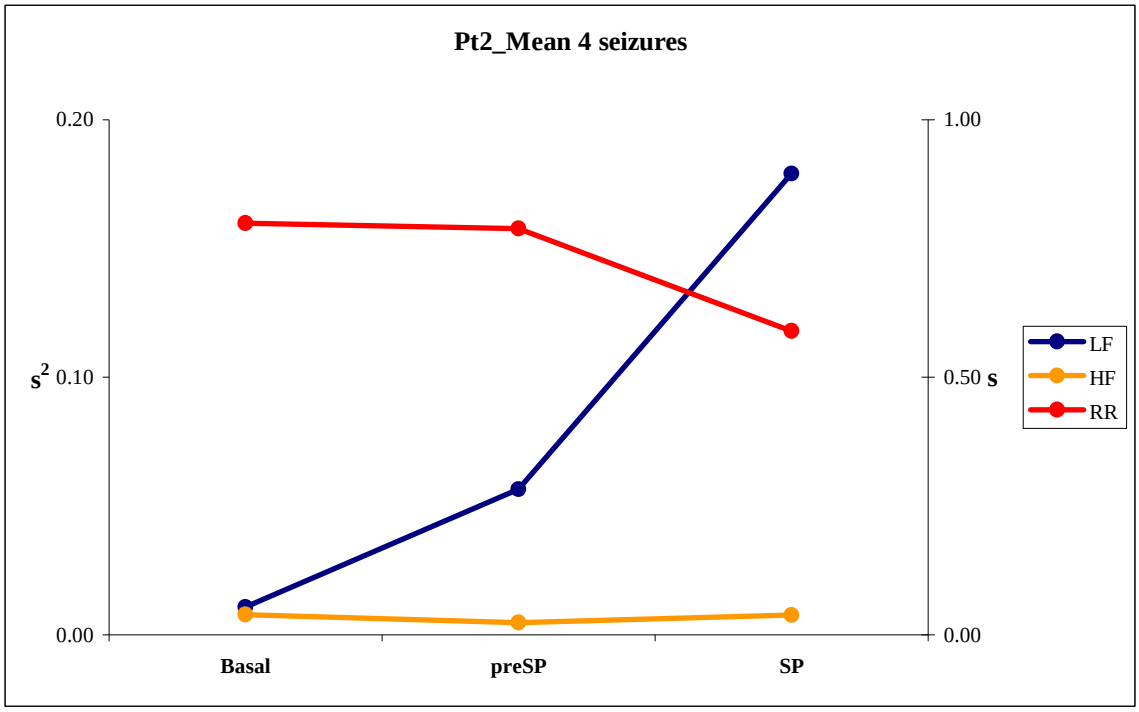


Figure 7.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Seizure Period	F=110.4 p<0.0001	B=-0.03 p=0.078	yes	B=-0.255 p<0.0001	yes
HF	-	-	-	-	-	-
LF	Seizure Period	F=145.2 p<0.0001	B=0.486 p<0.0001	yes	B=1.210 p<0.0001	yes

Table 7. Single subject analysis of patient 2. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

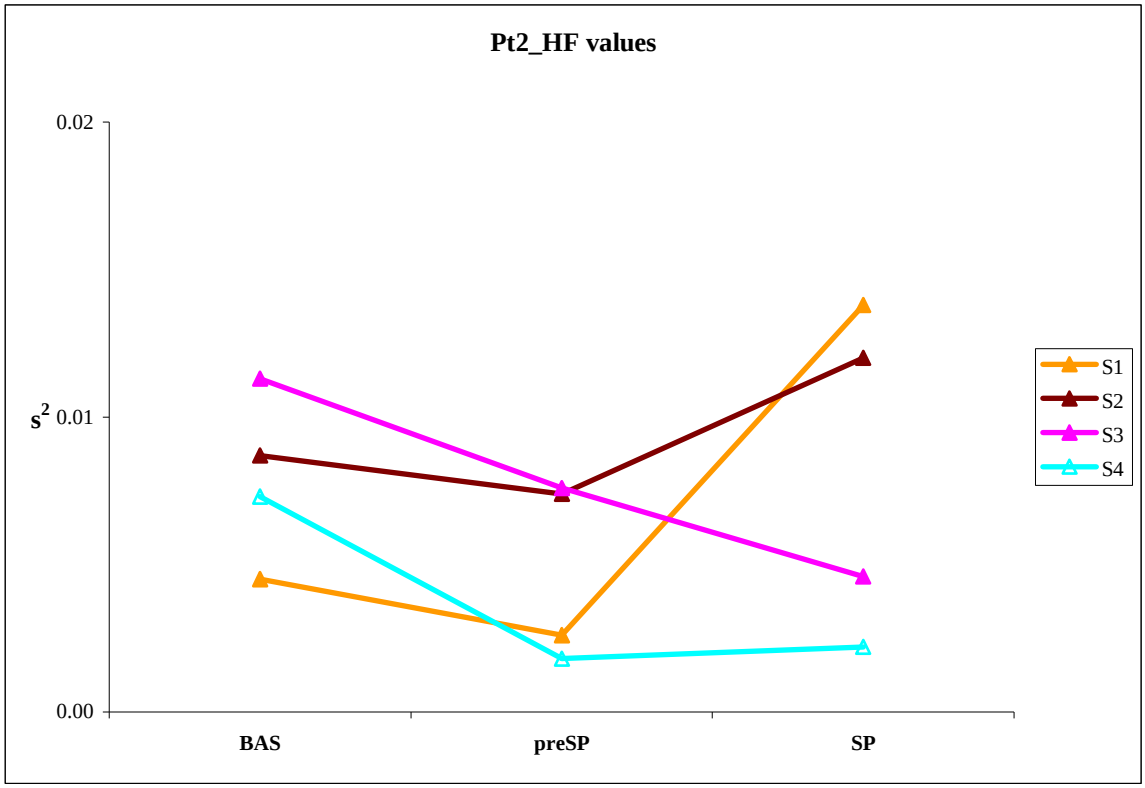


Figure 8. The pattern of changes in mean values of HF differed in the four seizures analyzed.

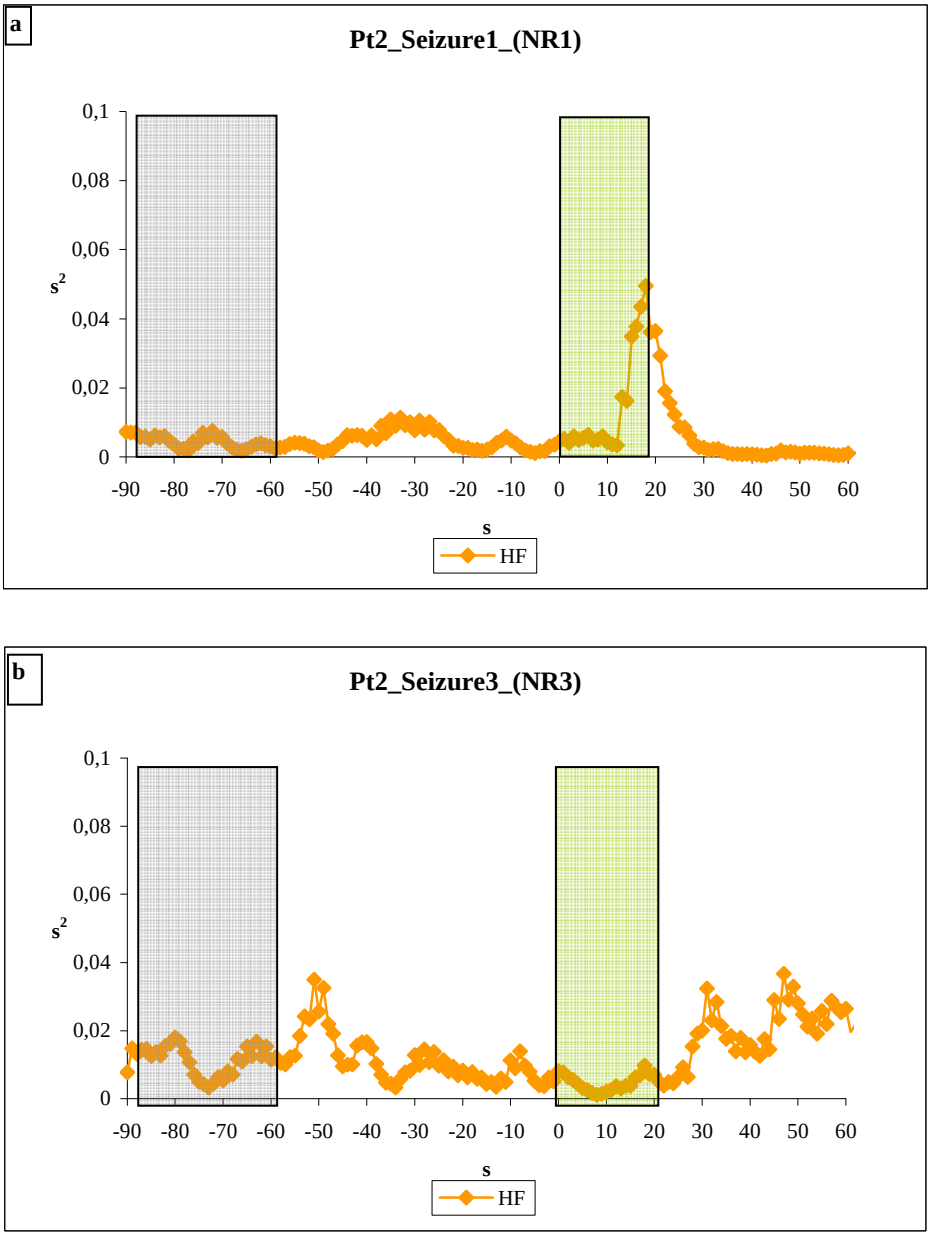
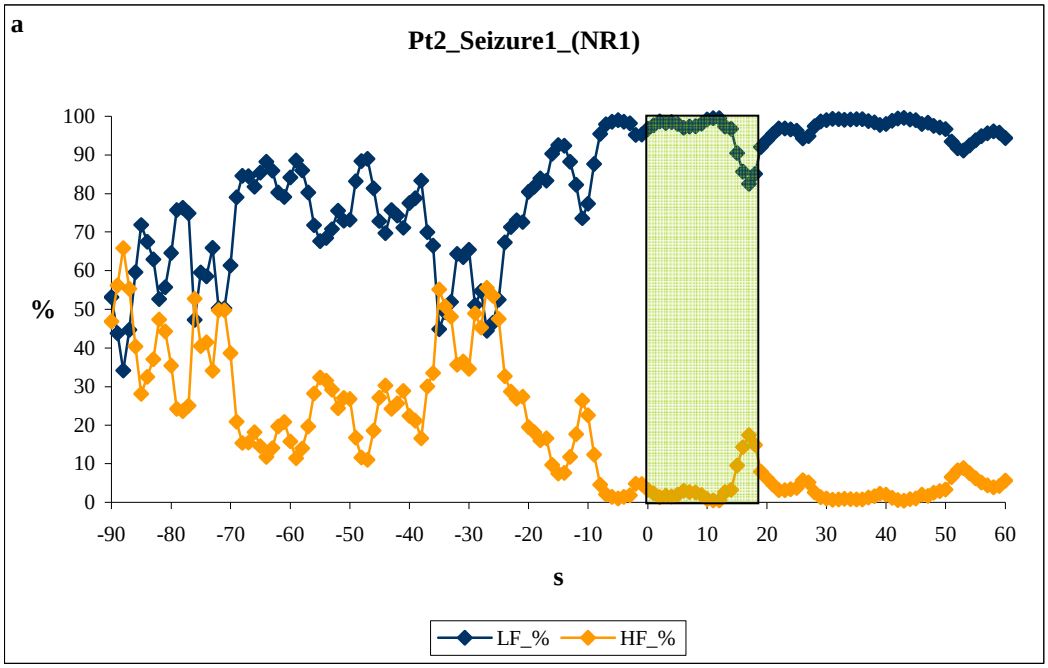


Figure 9. HF values during seizure 1 (a; green square: SP=18 s) and seizure 3 (b; green square: SP=23 s) of Patient 2. Compared to basal values (grey square: from -90 to -60 s), HF remained unchanged **(a)** or decreased **(b)** in the first part of the SP (from 0 to 10 s). In the second part of the SP, HF significantly increased only in seizure 1**(a)**.



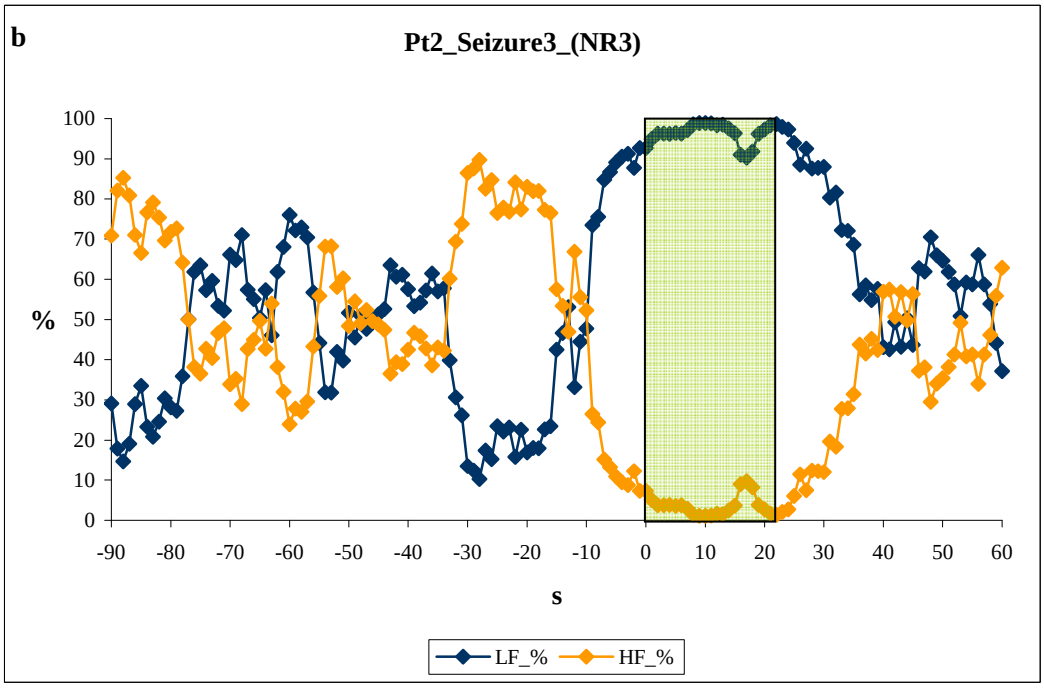


Figure 10. Normalized values of HF and LF during seizure 1 (**a**; seizure period=18 s) and seizure 3 (**b**; seizure period=23 s) of Patient 2. The same pattern of HF% and LF% changes were observed during the seizure period (green square) in both seizures.

4.3.3. Patient 3

Mean RRi, HF and LF values of the eight seizures analyzed are reported in Figure 11. Results of single subject analysis for this patient were in accordance with the group analysis for RRi and LF values (Table 8). Single subject analysis of HF values was not performed due to the different patterns of changes observed in different seizures (Figure 12). However, as for patient 2, an increase in LF% and a decrease of HF% was observed during the seizure period of all the seizures.

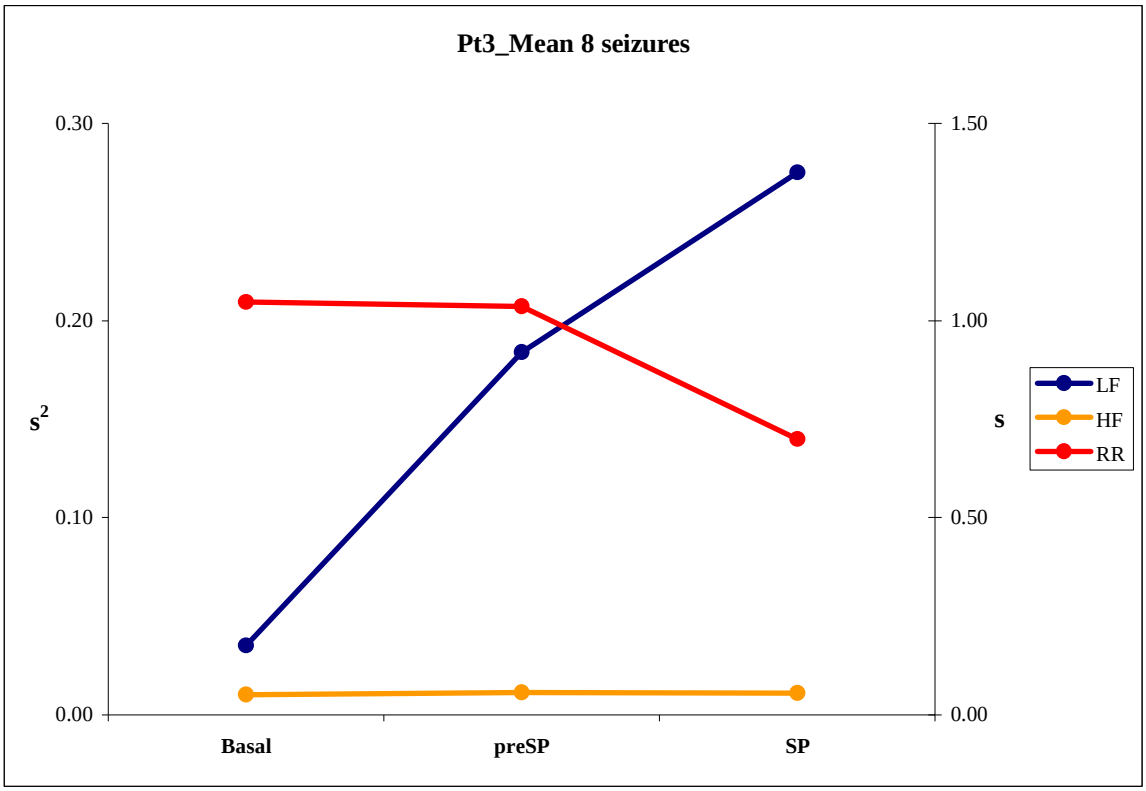


Figure 11.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Seizure Period	F=100.3 p<0.0001	B=-0.005 p=0.838	y	B=-0.181 p<0.0001	y
HF	-	-	-	-	-	-
LF	Seizure Period	F=51.4 p<0.0001	B=0.562 p<0.0001	y	B=0.824 p<0.0001	y

Table 8. Single subject analysis of patient 3. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis; **y:** yes; **n:** no.

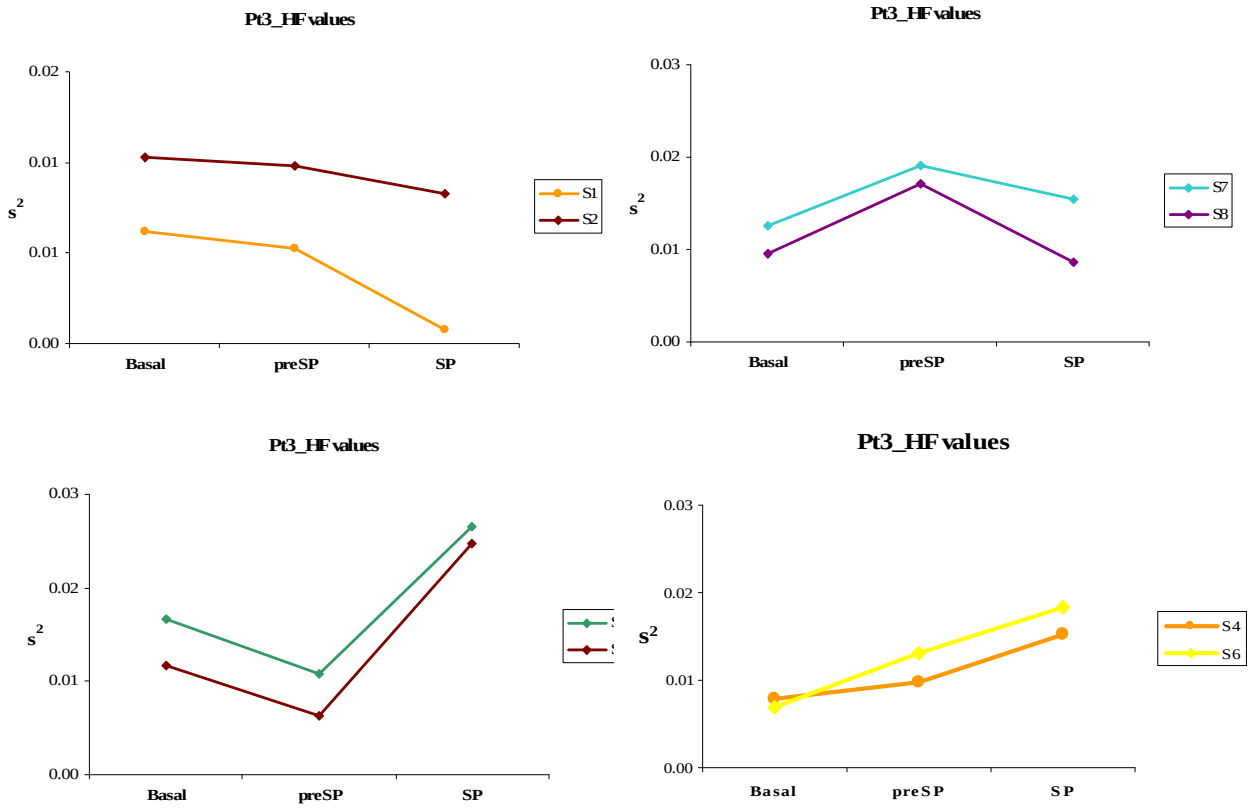


Figure 12. Means of HF values during the three defined periods of different seizures of Patient 3. Four different patterns of HF changes were observed. Only two seizures, lasting>10 s (S1; S2), presented HF changes similar to the pattern observed in the group analysis.

4.3.4. Patient 4

Mean RRi, HF and LF values of the three seizures during the three different periods are plotted in Figure 13. Single subject analysis for this patient found an accordance with the results of the group analysis for RRi and LF values. For HF values accordance was present only for the SP (Table 9). Compared with basal values, HF values significantly decreased also in the preSP.

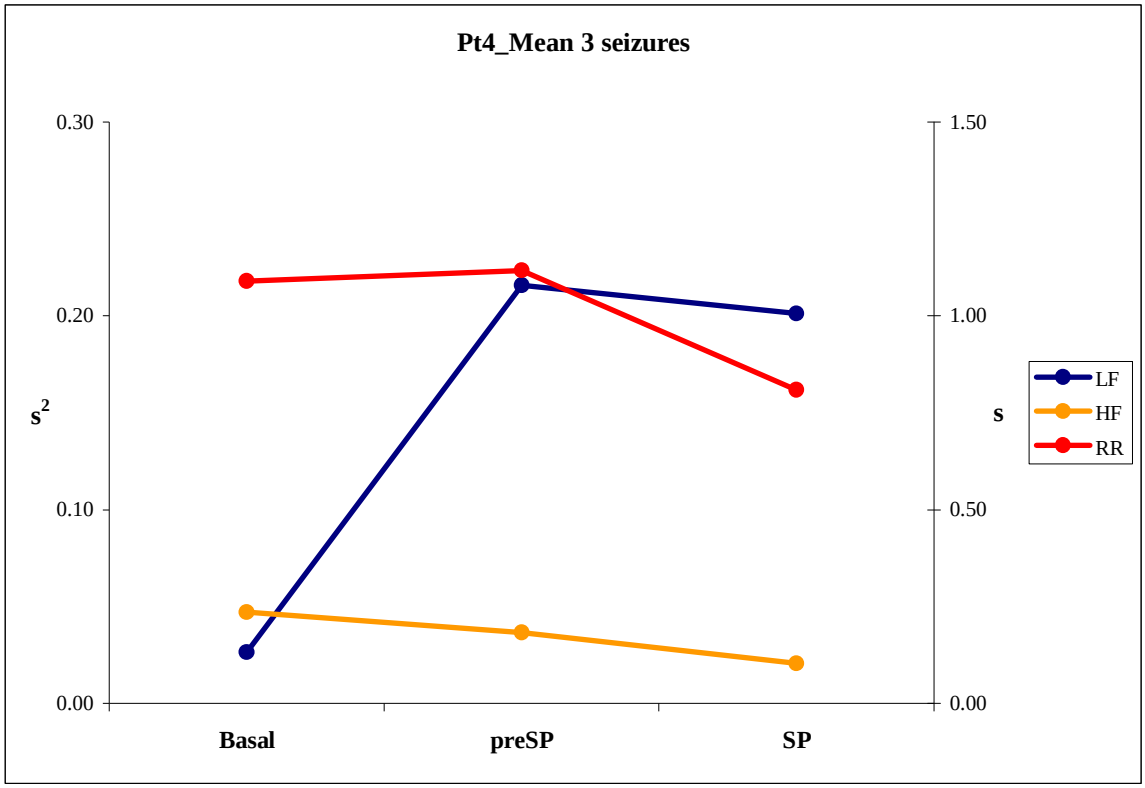


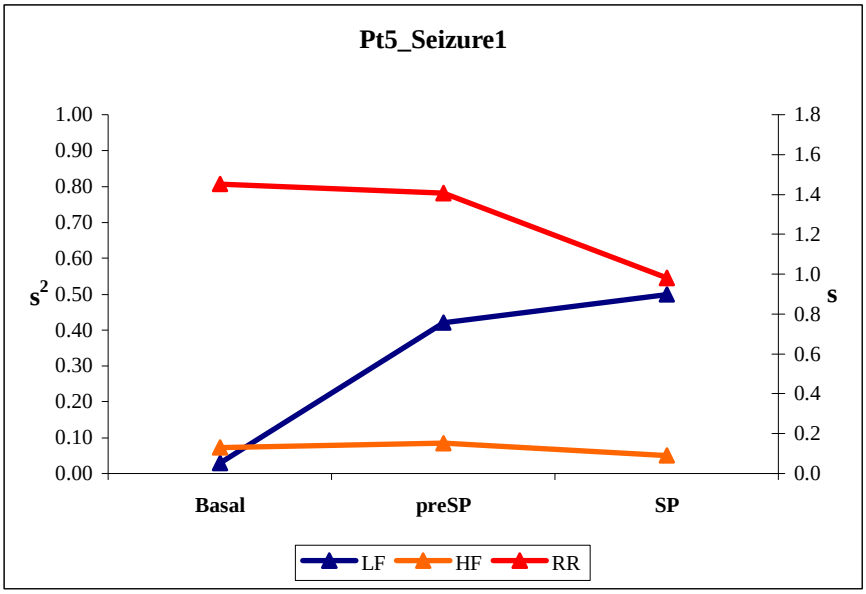
Figure 13.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Period	F=162 p<0.0001	B=0.028 p=0.104	yes	B=-0.281 p<0.0001	yes
HF	Seizure Period	F=31.1 p<0.0001	B=-0.212 P=0.003	no	B=-0.858 p<0.0001	yes
LF	Seizure Period	F=94.8 p<0.0001	B=0.910 p<0.0001	yes	B=0.991 p<0.0001	yes

Table 9. Single subject analysis of patient 4. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

4.3.5. Patient 5

Mean RRi, HF and LF values of the two seizures of patient 5 during the three different periods are plotted in Figure 14. Single subject analysis for this patient was not performed as only two seizures were analyzed. Patterns of RRi, HF and LF visually reproduced the results obtained in the group analysis except for the RRi values of seizure 2 that started to decrease in the preSP.


Pt5_Seizure1
1.00
0.90
0.80
0.70
0.60
s2 0.50
0.40
0.30
0.20
0.10
0.00
1.8
1.6
1.4
1.2
1.0
s
0.8
0.6
0.4
0.2
0.0
Basal
preSP
SP
LF
HF
RR

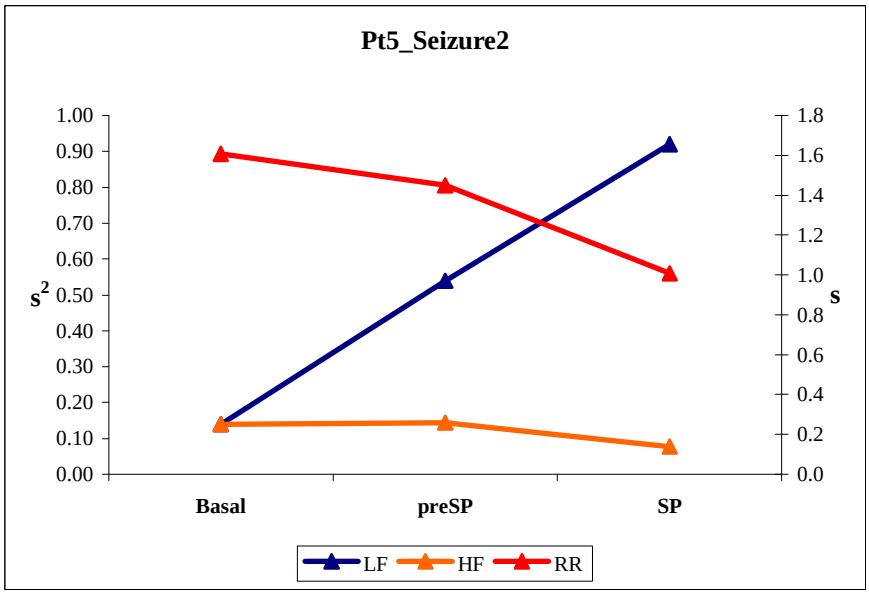

Pt5_Seizure2
1.00
0.90
0.80
0.70
0.60
s2 0.50
0.40
0.30
0.20
0.10
0.00
1.8
1.6
1.4
1.2
1.0
s
0.8
0.6
0.4
0.2
0.0
Basal
preSP
SP
LF
HF
RR

Figure 14.

4.3.6. Patient 6

Mean RRi, HF and LF values of the three seizures of patient 6 during the three different periods are plotted in Figure 15. Single subject analysis for this patient was in agreement with the results of the group analysis for RRi and HF values. For LF values accordance was present only for the preSP (Table 10), whereas LF was significantly decreased in the SP. However, this is probably due to the fact that the marked increase in HR, reflecting high sympathetic activation, was accompanied by a reduction in total power of HRV spectral components (Figure 16). After plotting normalized units of LF and HF, an increase in LF% during seizures became evident (Figure 17). As previously explained, this patient presented seizures lasting longer than three minutes comprising a first part (SP_1) with hyperkinetic automatism and a second part (SP_2) characterized by a clear EEG epileptic discharge in the left centro-fronto-temporal regions (Figure 18) associated with behavioural arrest, unresponsiveness and assumption of an asymmetric posturing of the right arm. A marked decrease of RRi and an increase in LF% were observed during both SP_1 and SP_2.

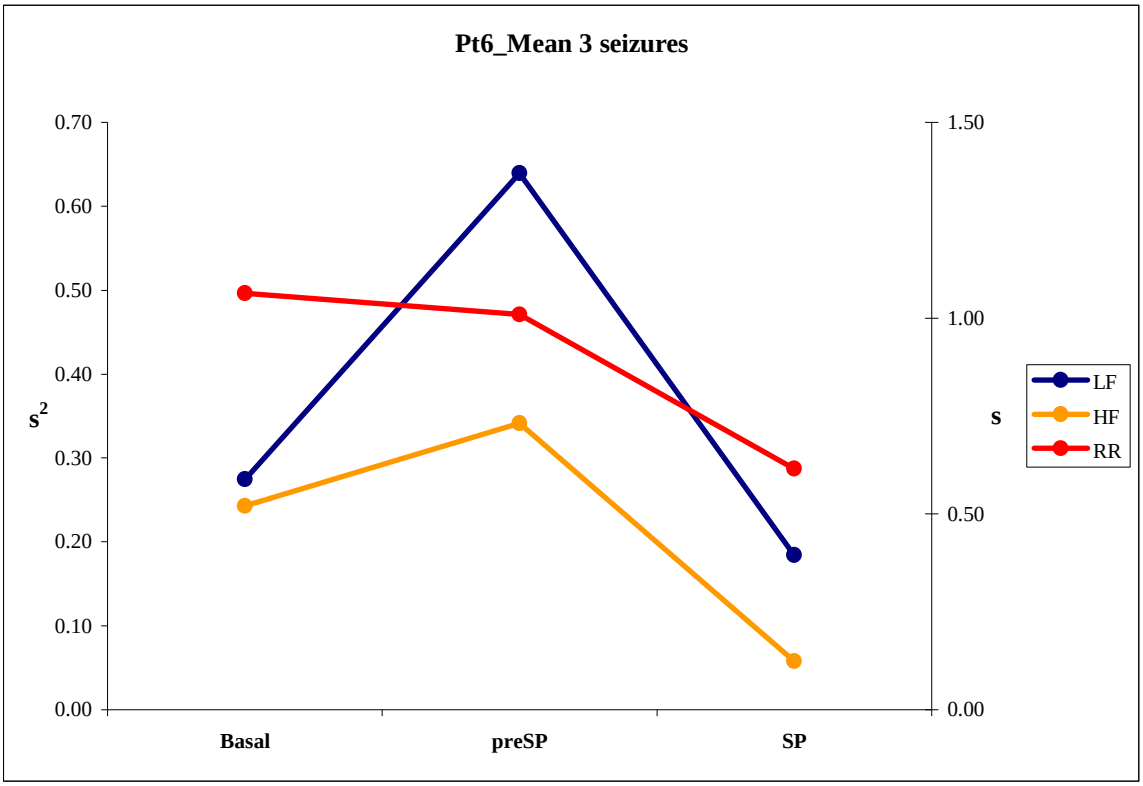


Figure 15.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Seizure Period	F=232.1 p<0.0001	B=0.0.038 p=0.327	yes	B=-0.478 p<0.0001	yes
HF	Period	F=128.9 p<0.0001	B=0.107 P=0.575	yes	B=-1.412 p<0.0001	yes
LF	Seizure Period	F=17.1 p<0.0001	B=0.782 p<0.0001	yes	B=-0.220 p<0.010	no

Table 10. Single subject analysis of patient 6. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

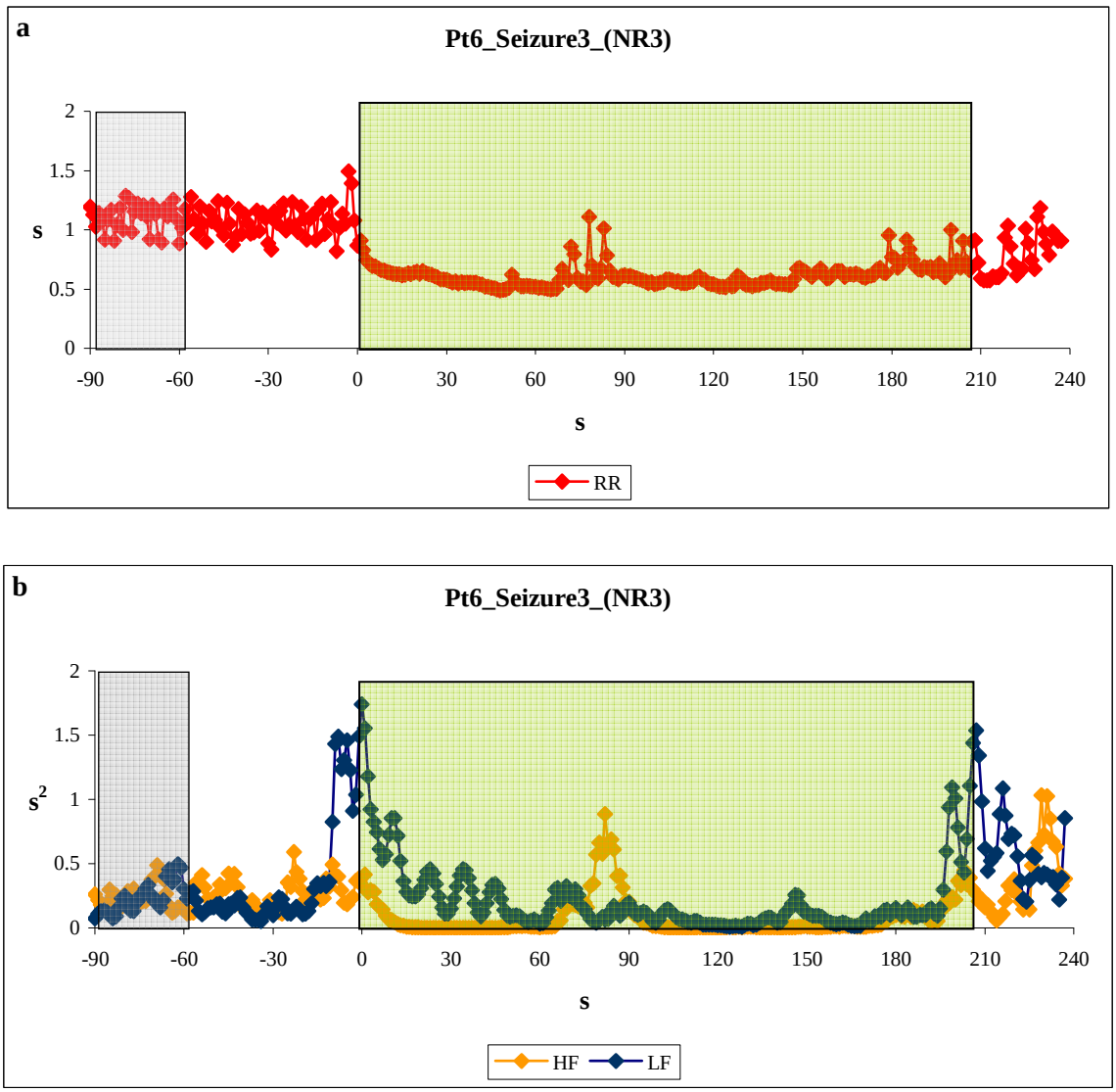


Figure 16. Changes in RRi **(a)**, LF and HF absolute values **(b)** during seizure 3 of patient 6. Compared to the basal period (grey square: from -90 to -60 s), RRi, LF and HF absolute values significantly decreased during the first (SP1: from 0 to 130 s) and second parts (SP2: from 130 to 210 s) of the seizure period (green square: from 0 to 209 s).

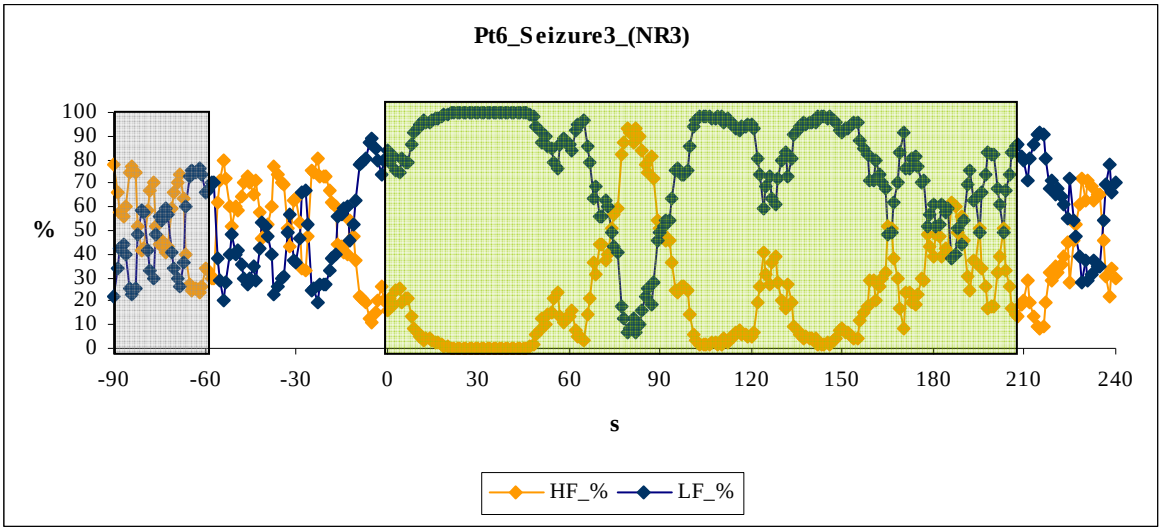


Figure 17. LF and HF expressed in normalized units during seizure 3 of patient 6. Compared to the basal period (grey square: from -90 to -60 s), LF% significantly increased during the first (SP1: from 0 to 130 s) and second parts (SP2: from 130 to 210 s) of the seizure period (green square: from 0 to 209 s) suggesting a marked sympathetic activation.

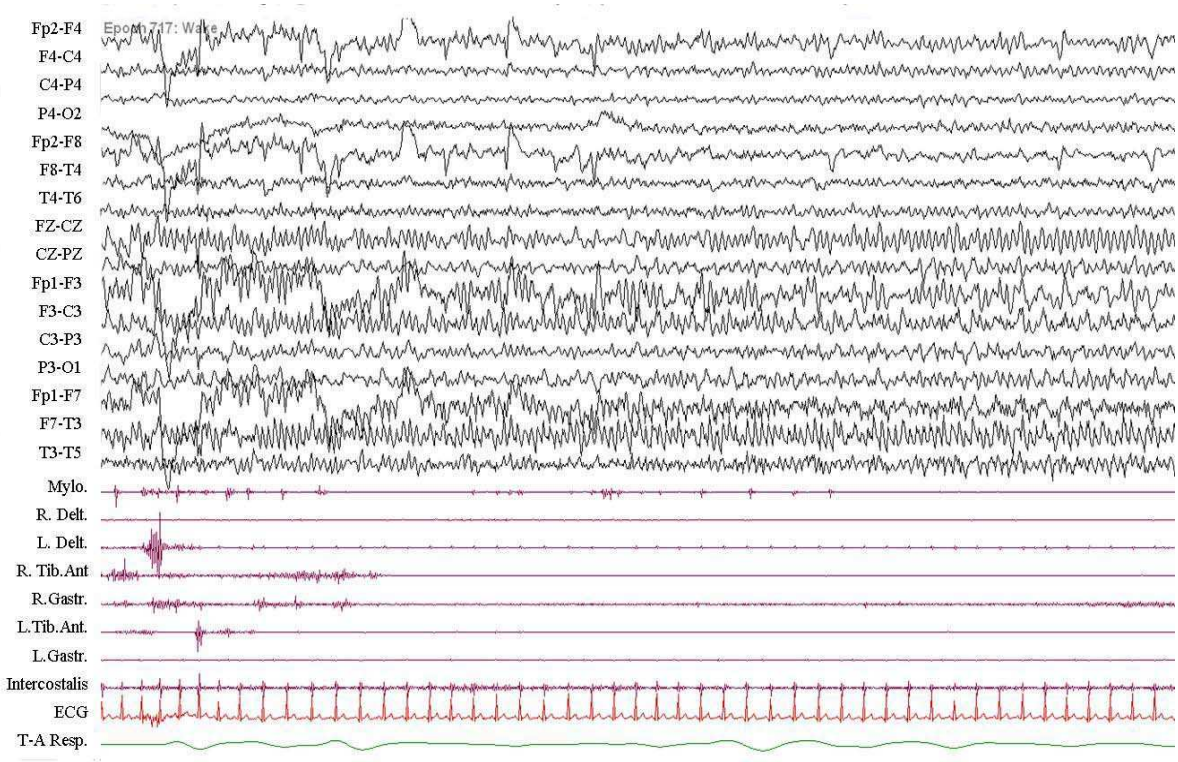


Figure 18. Polygraphic recording of seizure 3 of patient 6 (speed 30 mm/s). 130 s after clinical onset of the seizure a clear epileptic discharge was evident in the centro-fronto-temporal derivations. During the discharge the patient presented behavioural arrest, unresponsiveness and raised his right arm in a dystonic posturing. Although few movements occurred, tachycardia was observed.

EEG (Fp2-F4, F4-C4, C4-P4, P4-O2, Fp2-F8, F8-T4, T4-T6, Fz-Cz, Cz-Pz, Fp1-F3, F3-C3, C3-P3, P3-O1, Fp1-F7, F7-T3, T3-T5); R.: right; L.: left; Mylo.: mylohyoideus muscle, Delt.: deltoideus muscle; Gastr: gastrocnemius muscle; Tib. Ant.: tibialis anterior muscle; Intercostalis muscle; ECG: electrocardiogram (from a standard D2 lead); T-A Resp.: thoracic and abdominal efforts (strain gauge).

4.3.7. Patient 7

Mean RRi, HF and LF values of the seven seizures of patient 7 during the three different periods are plotted in Figure 19. As for patient 6, single subject analysis for patient 7 was in agreement with the results of the group analysis for RRi and HF values. For LF values accordance was present only for the preSP (Table 11), whereas LF was significantly decreased in the SP. Normalized units of LF and HF were again plotted to better explain the sympathetic-parasympathetic control during the seizure (Figure 20).

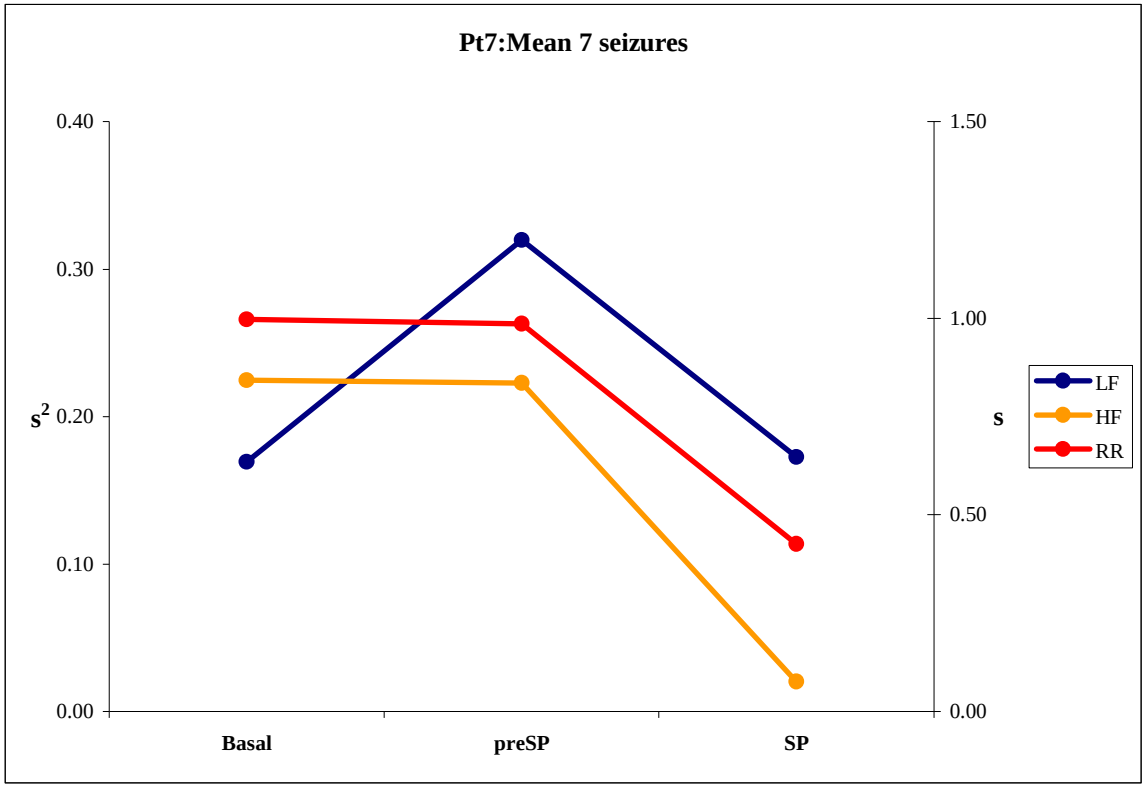


Figure 19.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Period	F=1581 p<0.0001	B=-0.12 p=0.475	yes	B=-0.571 p<0.0001	yes
HF	Seizure Period	F=382.2 p<0.0001	B=-0.035 P=0.778	yes	B=-2.152 p<0.0001	yes
LF	Period	F=30 p<0.0001	B=0.226 P=0.001	yes	B=-0.225 p<0.0001	no

Table 11. Single subject analysis of patient 7. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

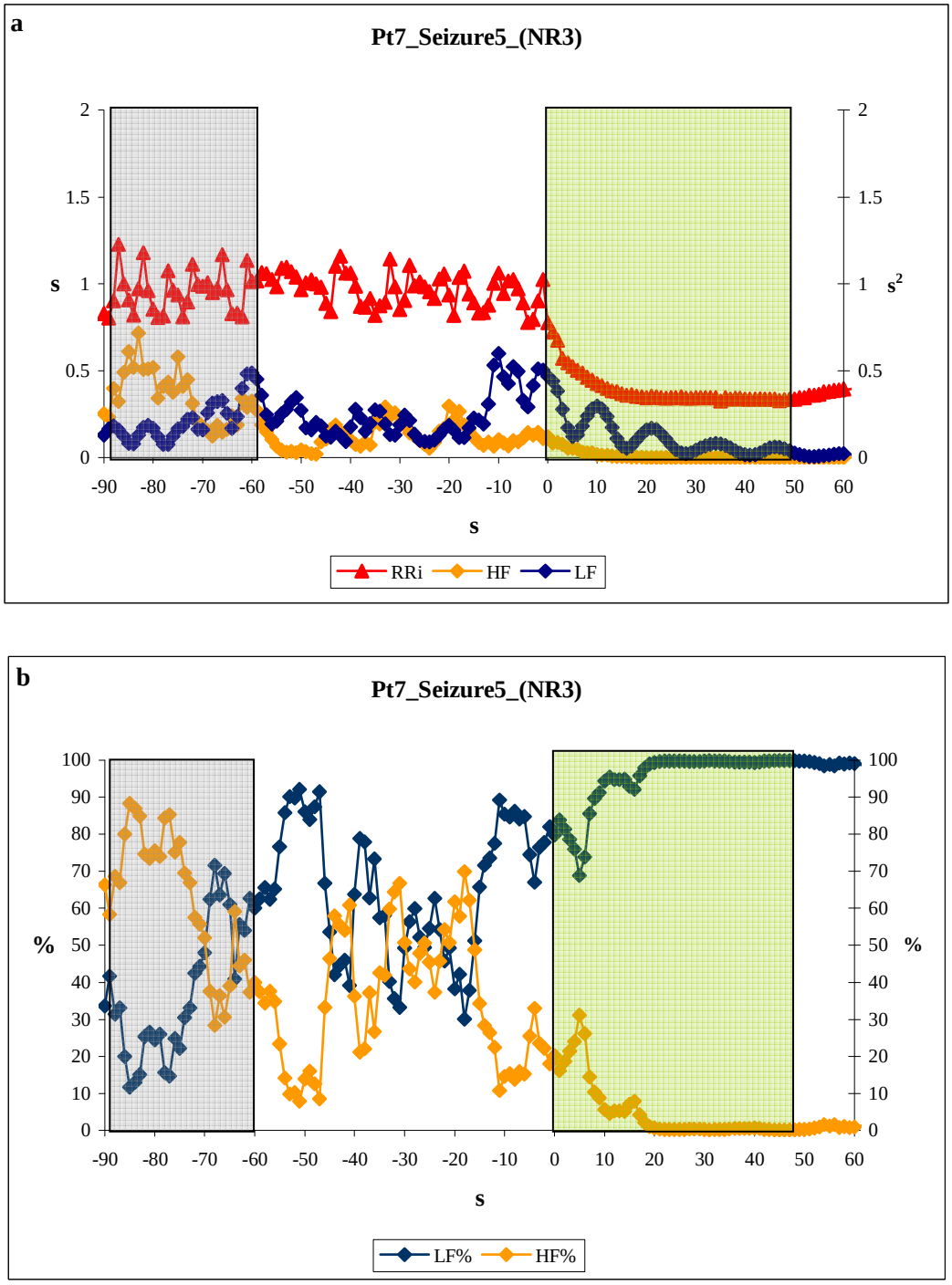


Figure 20. a) Compared to the basal period (grey square: from -90 to -60 s), a significant decrease of RRi and absolute values of LF and HF were observed during the seizure period (green square: from 0 to 60 s). **b)** Compared to basal values, LF% increased during the seizure period.

4.3.8. Patient 8

Mean RRi, HF and LF values of the seven seizures of patient 8 during the three different periods are plotted in Figure 21. Single subject analysis for this patient was in agreement with the results of the group analysis for RRi and LF values (Table 12). Compared to basal values, values of HF significantly decreased in both the preSP and the SP.

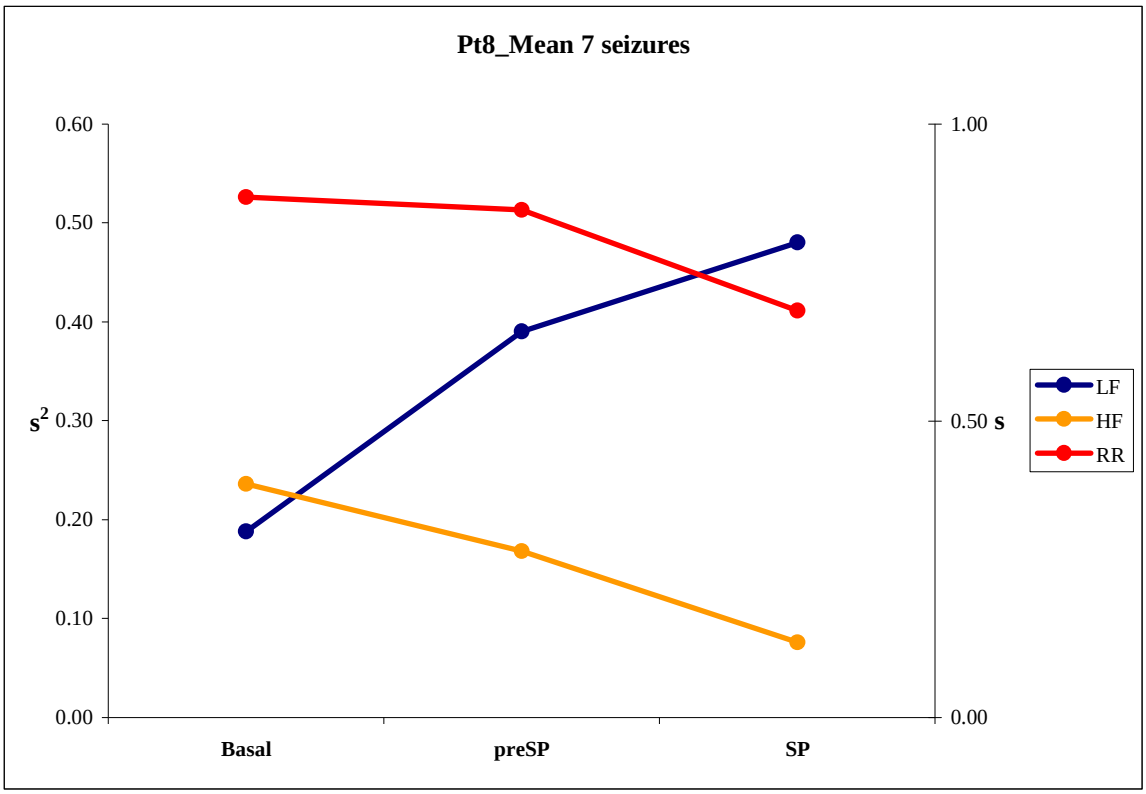


Figure 21.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	Agreement with the GA	SP vs Basal (B; p values)	Agreement with the GA
RRi	Period	F=130 p<0.0001	B=0.021 p=0.172	yes	B=-0.191 p<0.0001	yes
HF	Seizure Period	F=30.2 p<0.0001	B=-0.291 P=0.002	no	B=-0.401 p<0.0001	yes
LF	Seizure Period	F=36.4 p<0.0001	B=0.512 p<0.0001	yes	B=0.420 p<0.0001	yes

Table 12. Single subject analysis of patient 8. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors; **GA:** group analysis.

4.3.9. Summary of the single subject analysis

Single subject analysis was performed in seven out of eight patients as only two seizures had been analyzed for patient 5 (Table 13). This analysis confirmed the results obtained in the group analysis for RRi changes during the preSP and the SP in all subjects analyzed. For HF variations complete accordance with the group analysis was found for the SP in five patients and for the preSP in three. Analysis was not performed in patients 2 and 3 as a similar pattern of HF changes was not visually observed in the majority of their seizures. Lastly, single subject analysis confirmed the results obtained for LF changes during the preSP in seven patients and during the SP in five patients. In two patients LF absolute values were decreased during the SP due to the reduction in total power of spectral components as demonstrated after plotting the normalized units.

Patient	**RRi**		**HF**		**LF**	
	preSP	**SP**	**preSP**	**SP**	**preSP**	**SP**
1	yes	yes	yes	yes	yes	yes
2	yes	yes	-	-	yes	yes
3	yes	yes	-	-	yes	yes
4	yes	yes	no	yes	yes	yes
6	yes	yes	yes	yes	yes	no
7	yes	yes	yes	yes	yes	no
8	yes	yes	no	yes	yes	yes

Table 13. Agreement between the results of the single subjects analysis and the group analysis for the parameters analyzed.

4.4. PATIENT 9: A CASE OF NOCTURNAL ICTAL ASYSTOLE

4.4.1. History and recordings

Patient 9, a 40-year-old right-handed woman born in twin delivery, with a positive family history for sleep talking, began having seizures at 17 years of age. Seizures occurred exclusively during sleep, lasted a few minutes, and were characterized by vocalizations and uncoordinated and violent movements involving the axial musculature and the limbs. Urinary incontinence was sometimes associated. Subjectively she was usually unaware of the episodes but she occasionally reported feeling as if "the skin around the right eye was stretching" during the night. Response to CBZ (400 mg/die) was initially favourable with complete control of the seizures until 35 years of age. At that time seizures reappeared with the same semeiological features and low frequency (less than one per month). However one year before our evaluation, seizure frequency had increased to several episodes nightly despite CBZ at the same dosage. Patient also had hypothyroidism, treated with levothyroxine, and chronic daily headache. Neurological examination was normal. Brain MRI disclosed thickening of the left amygdala and ***blurring*** of the grey–white matter junction on T2-weighted images in the left mesial temporal regions.

A whole night VPSG (*Connex EEG Sleep-XLTEK Software 5.4;* sampling rate 256 Hz) and 24-h video polygraphic monitoring (*Nihon Kohden EEG-1200*; sampling rate 500 Hz) were performed. Polygraphic recordings included standard bipolar EEG (according to the International 10-20 System), right and left electrooculogram, surface electromyogram (EMG) of mylohyoideus, bilateral deltoideus and tibialis anterior muscle, ECG (from a standard D2 lead), microphone, oro-nasal (thermistor), thoracic and abdominal respirograms (strain gauge) and oxygen saturation (pulse oxymeter).

During wakefulness interictal EEG revealed normal activity while epileptic abnormalities with sharp wave features involving the anterior vertex and the centro-parietal regions of both the hemispheres were observed during sleep. Three seizures were recorded during two separate nights associated with ictal asystole (IA; mean duration: 18±4 s) and characterized by similar motor features. All the seizures arose from deep NREM sleep.

At the beginning of the seizure the patient presented a downward contraction of the rima oris followed by oroalimentary automatisms associated with movement of the pelvis in an anterior-posterior direction. After this she assumed an asymmetric tonic posturing with extension of the right arm and leg, while performing manual automatisms with the left hand. Repetitive screams and hyperkinetic movements of both the superior and inferior limbs were then observed before the end of the seizure.

A diffuse flattening of the EEG activity appeared nearly 20 seconds before clinical seizure onset, followed by a diffuse fast, low voltage rhythmic activity that persisted until the first movement occurred and was associated with an increase in heart and respiratory rates (Figure 22). Muscular artefacts masked the EEG traces after seizure onset and during IA. Tachycardia persisted in the first part of the seizure and was followed by progressive bradycardia until IA occurred (see Figure 23 and RRi analysis). During IA the patient maintained the asymmetric tonic posturing and about two seconds before the end of IA she screamed and presented manual automatisms with the left hand. After IA, tachycardia was observed while normal HR was regained after the end of the seizure. After polygraphic recordings, the patient underwent a 24h ECG monitoring that showed normal results.

4.4.2. Analysis of cardiovascular parameters

Measurement of the RRi interval, spectral analysis of HRV by WT, and single subject statistical analysis were performed as explained in the methods section.

Four periods were selected to evaluate time-dependent cardiovascular changes during seizures before IA: basal period (Basal - 90 s to 60 s before the clinical seizure onset), pre-seizure period (preSP - 20 s preceding seizure), the first part of the seizure period (SP_1 – from clinical onset to the shortest RRi interval value reached before RRi started to return to basal values) and the second part of the seizure period (SP_2: from end of SP_1 to beginning of IA) (Table 14 and Figure 24).

Compared to basal values a significant decrease of RRi and HF absolute values, during both the preSP and SP_1 ($p<0.001$) and a significant increase during SP_2 ($p<0.001$) were observed, while a significant increase in LF absolute values was detected in preSP , SP_1 and SP_2 ($p<0.001$) (Table 15).

The pattern of RRi changes was confirmed in all three seizures during preSP and SP_1 and in two seizures during SP_2. In seizure 1 RRi progressively increased during SP_2, but the mean value of RRi remained decreased with respect to basal values as IA was reached in a shorter time (Table 14 and Figure 25). An increase in LF absolute values during the three periods was observed in all three seizures, whereas pattern of HF component changes was different only in preSP of seizure 3 during which HF values increased compared to basal values. Lastly, a significant increase in all three parameters (RRi, HF and LF absolute values) was observed in SP_2 compared to SP_1 ($p<0.0001$) (Figure 25).

4.4.3. Summary of cardiovascular changes in patient 9

During the preSP and in the first part of SP (12, 16, 14 s respectively) a significant tachycardia was observed associated with an increased sympathetic activity (increased LF absolute values and LF%). In the second part of the SP (9, 20, 13 s respectively) a progressive decrease in HR that gradually exceeded basal values occurred before IA. Bradycardia was associated with an increase in parasympathetic activity (increased HF absolute values and HF%) contrasted by a further increase in LF until the occurrence of IA. These data were consistent in the three seizures analyzed.

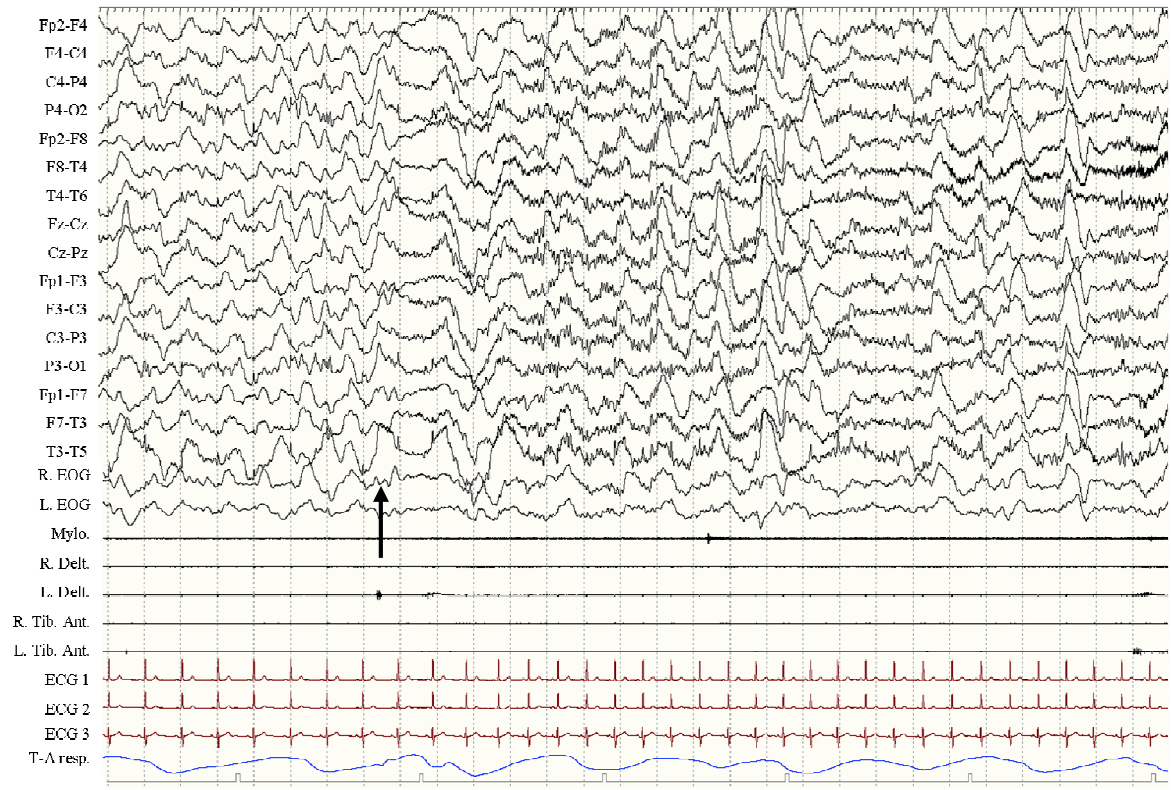


Figure 22. Polygraphic recording of seizure 2 of patient 9 (speed 30 mm/s). A diffuse flattening of the EEG activity (black arrow) was observed 20 seconds before the clinical onset, followed by a diffuse fast, low voltage rhythmic activity that persisted until the first movement occurred and was associated with an increase in heart and respiratory rae.

EEG (Fp2-F4, F4-C4, C4-P4, P4-O2, Fp2-F8, F8-T4, T4-T6, Fz-Cz, Cz-Pz, Fp1-F3, F3-C3, C3-P3, P3-O1, Fp1-F7, F7-T3, T3-T5); R.: right; L.: left; EOG: electrooculogram; Mylo.: mylohyoideus muscle; Delt.: deltoideus muscle; Tib. Ant.: tibialis anterior muscle; ECG: electrocardiogram (from a standard D2 lead); T-A resp.: thoracic and abdominal efforts (strain gauge).

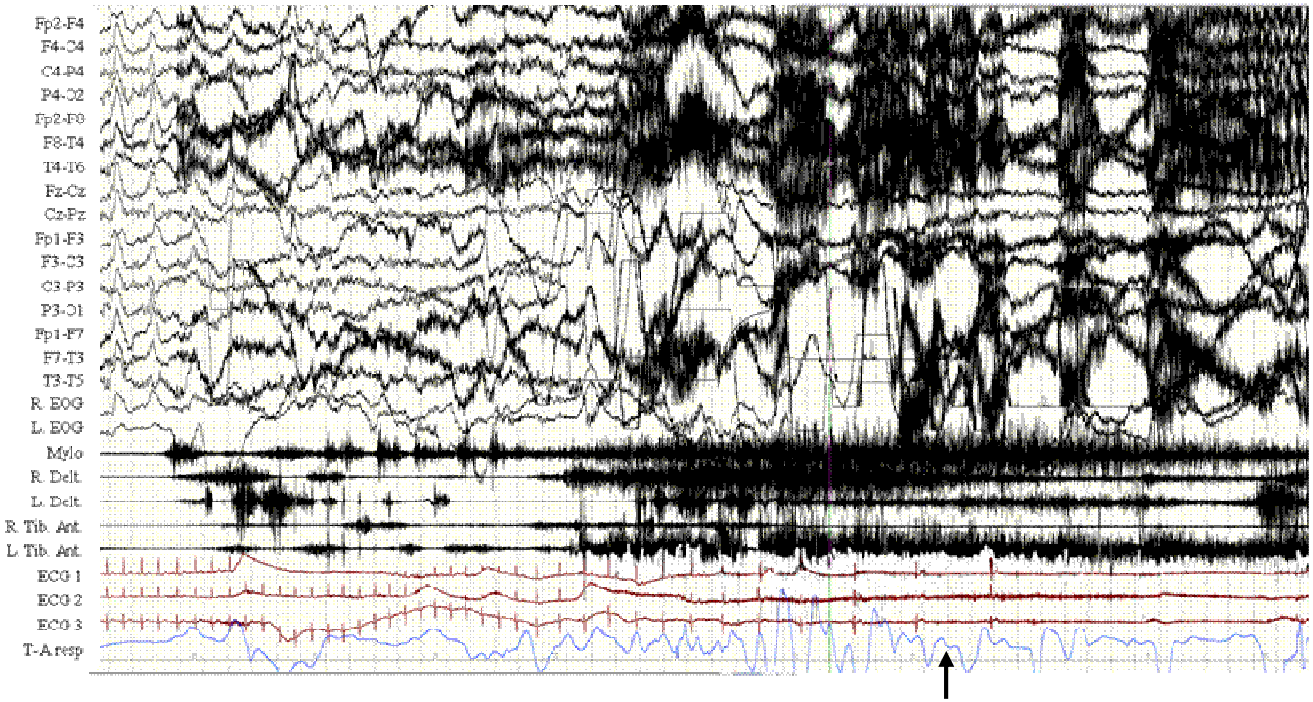


Figure 23. Polygraphic recording of seizure 2 of patient 9 (speed 30 mm/s). Tachycardia was observed after the clinical onset of the seizure and was then followed by progressive bradycardia until IA occurred. EEG (Fp2-F4, F4-C4, C4-P4, P4-O2, Fp2-F8, F8-T4, T4-T6, Fz-Cz, Cz-Pz, Fp1-F3, F3-C3, C3-P3, P3-O1, Fp1-F7, F7-T3, T3-T5); R.: right; L.: left; EOG: electrooculogram; Mylo.: mylohyoideus muscle; Delt.:deltoideus muscle; Tib. Ant.: tibialis anterior muscle; ECG: electrocardiogram (from a standard D2 lead); T-A resp.: thoracic and abdominal efforts (strain gauge

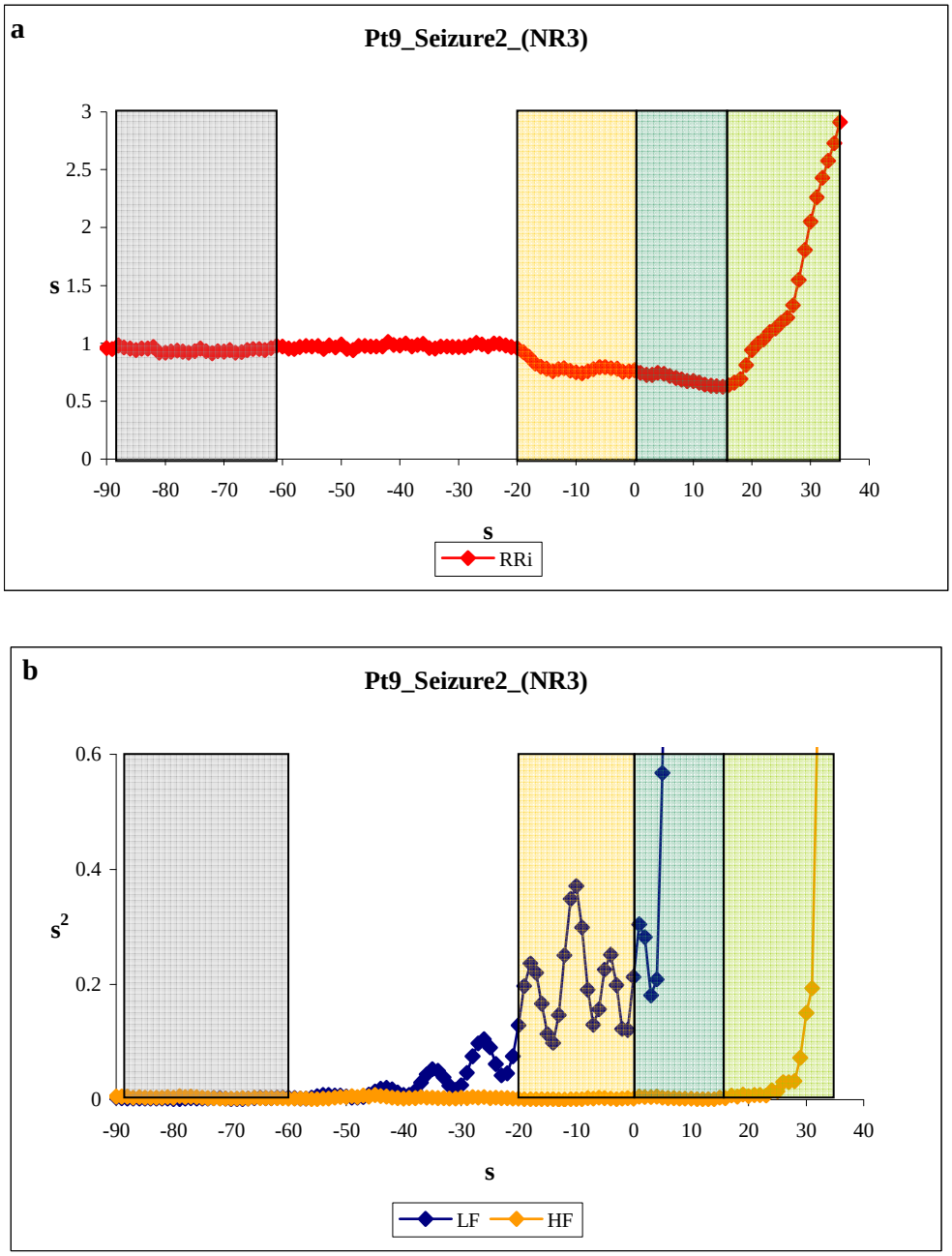


Figure 24. Changes in RRi **(a)**, LF and HF absolute values **(b)** during seizure 2 in patient 9 before IA. Basal period (grey square: BAS- from 90 s to 60 s before the clinical seizure onset), pre-seizure period (orange square: preSP- 20 s preceding seizure), first part of the seizure period (light blue square: SP_1 - from clinical onset to the shortest RRi interval value reached before RRi started to return to basal values) and second part of the seizure period (green square SP_2: from end of SP_1 to the beginning of IA).

Night	Time of seizure occurrence	SP_1 (s)	SP_2 (s)	AI (s)	postIA (s)	Seizure duration (s)
1	4.42.27	12	9	21	47	89
2	1.59.29	16	20	14	47	97
2	4.40.31	14	13	18	51	96

Table 14. SP_1: seizure period from clinical onset to the shortest RRi interval value reached before RRi started to return to basal values; **SP_2**: seizure period from the end of SP_1 to the beginning of IA; **IA**: ictal asystole; **postIA**: from the end of IA to the end of the seizure.

Dependent Variable	Fixed factors	F; p of the model	PreSP vs Basal (B; p values)	SP_1 vs Basal (B; p values)	SP_2 vs Basal (B; p values)
RRi	Period	F=41.3 p<0.0001	B=-0.204 p<0.0001	B=-0.357 p<0.0001	B=0.283 p<0.0001
HF	Period	F=120.5 p<0.0001	B=-0.490 p<0.0001	B=-0.423 p<0.0001	B=1.294 p<0.0001
LF	Seizure Period	F=301.7 p<0.0001	B=1.367 p<0.0001	B=1.961 p<0.0001	B=2.983 p<0.0001

Table 15. Single subject analysis of patient 9. **F**: propriety of the model expressed through Fisher's F; **B**: b coefficients for factors.

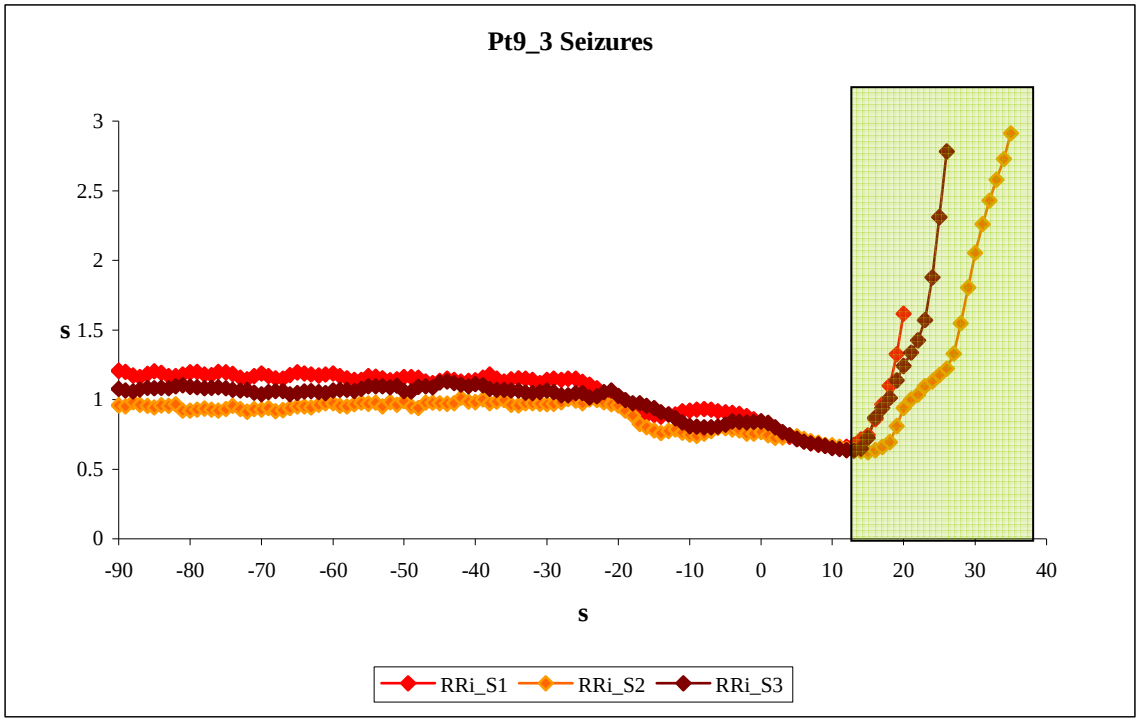


Figure 25. Changes in RRi during the three seizures of patient 9 before ictal asystole. Compared to the basal period (from 90 to 60 s before the clinical seizure onset) a significant decrease of RRi was observed during the preseizure period (20 s preceding seizure) and during the first part of the seizure period (SP_1 - from clinical onset to the shortest RRi interval value reached before RRi started to return to basal values), while in the second part of the seizure period (green square: from the end of SP_1 to the beginning of ictal asystole) progressive bradycardia occurred.

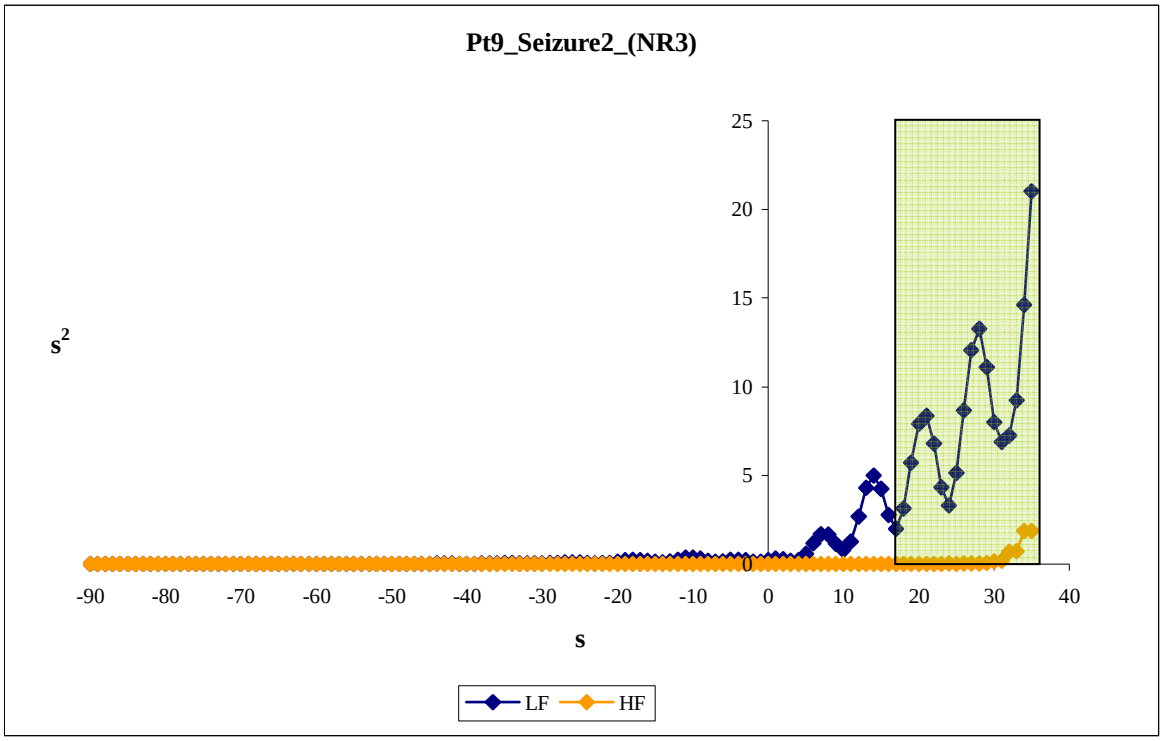


Figure 26. Changes in LF and HF absolute values during seizure 2 of patient 9 before ictal asystole (IA). Compared to the first part of the seizure period (from 0 to 15 s after clinical seizure onset) a significant increase in LF and HF absolute values was observed during the second part of the seizure period (green square: from 15 s to IA). In particular, LF continued to increase until IA occurred.

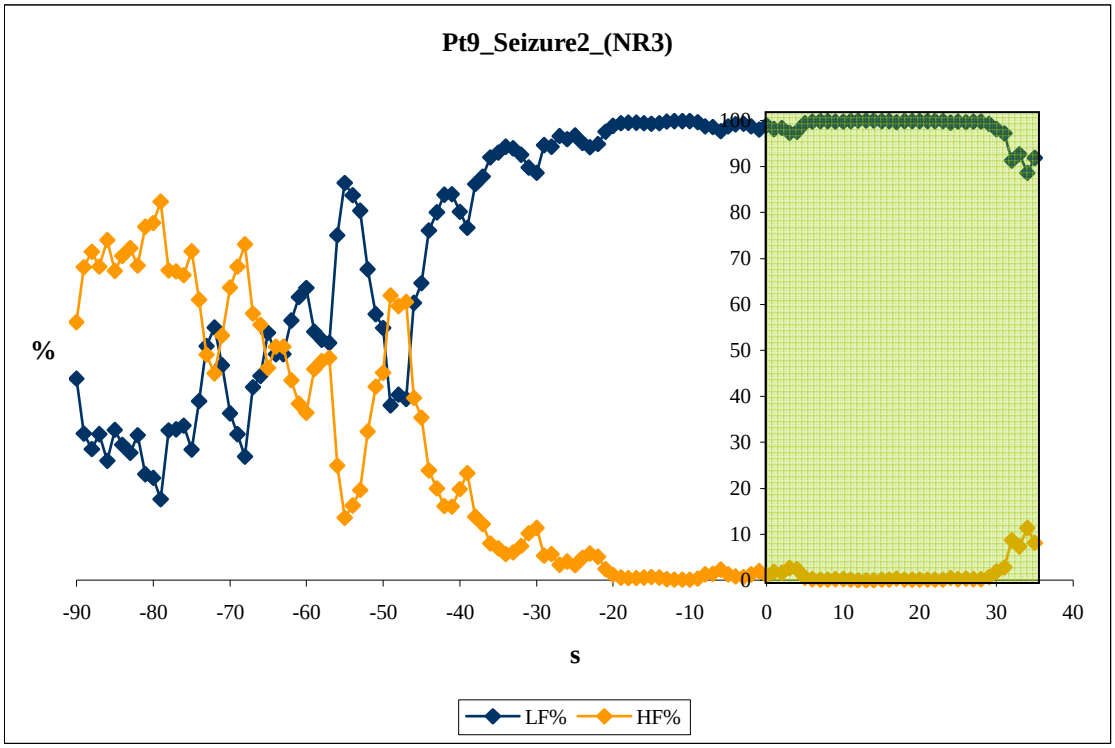


Figure 27. Changes in LF and HF expressed in normalized units during seizure 2 in patient 9 before ictal asystole. A marked predominance of the LF component was evident throughout the seizure period (green square), with only a slight reduction a few seconds before ictal asystole occurred.

5. DISCUSSION

5.1. CARDIOVASCULAR CHANGES DURING NFLE SEIZURES

This study was designed to investigate the evolution over time of changes in HR and in its sympathetic and parasympathetic control in relation to epileptic motor phenomena of sleep with clinical features of NFLE seizures. We were particularly interested in the temporal relationship between cardiac modifications and seizure onset. According to the hypothesis that epileptic cardiovascular manifestations result not only from ictal motor activity, but also reflect epileptic discharges involving regions of the central autonomic network (CAN), we sought to establish whether cardiovascular changes can be considered an early clinical sign and hence a reliable diagnostic marker of seizure occurrence.

Recordings with intracerebral electrodes [1, 2] and SPECT studies [3,4] demonstrated that during NFLE seizures the epileptic discharge could arise or involve regions of the CAN devoted to cardiovascular modulation, like the cingulate gyrus and the insular cortex.

In our study electroencephalographic investigations were performed through scalp electrodes and, as expected, failed to disclose and localize the onset of the epileptic discharge. For this reason we selected as seizure onset a clinical marker corresponding to the first movement recorded.

We included in the study nine patients (44 seizures) whose clinical and electrophysiological characteristics broadly matched those reported in a large population of NFLE patients [5]. The differences observed, like the higher prevalence of drug-resistant seizures or predominance of seizures with NPD features, are the consequence of our decision to apply strictly criteria for both patients and seizure selection, to prevent

inaccuracy in the definition of the epileptic nature of the motor phenomena. Moreover, the need to avoid confounding influences on HR due to coexistent cardiovascular or respiratory disorders limited the number of patients finally selected. Likewise the high frequency of minor motor events recorded in our patients and the tendency of seizures to recur periodically with a short time span [5, 6] led to the exclusion of several seizures (18 NPD; several PA in the eight patients finally included) to respect the minimum interval required between the seizure under analysis and a previous phenomenon arising from sleep.

The first important finding of our study is that a significant ($p<0.001$) increase in HR was observed during the seizure period in all the seizures analyzed (mean RRi values ± SD in the basal period: 0.984 ± 0.17 s ; in the SP: 0.604 ± 0.17 s). Only in one patient was tachycardia followed by progressive bradycardia and asystole, and for this reason we treated this patient separately.

These data are in accordance with previous studies investigating HR changes during partial seizures of different origin disclosing a high prevalence of ictal tachycardia [7-10]. They also confirmed the clinical observation of remarkable autonomic activation associated with seizures reported in a large series of NFLE patients [5].

Regarding the temporal relationship between cardiovascular modifications and seizures onset, we did not find significant HR changes before clinical seizure onset. This result was obtained both in the group analysis and in the single subject analysis for all patients analyzed. Previous studies reported that tachycardia preceded EEG seizure discharges by an average of 18.7 s in more than 75% of seizures evaluated with scalp electrodes and by an average of 11 s in 45% of seizures recorded with invasive subdural EEG [8, 10]. However, early onset tachycardia was detected more frequently in seizures arising from the temporal lobe, especially from mesial structures, compared with seizures of extratemporal

lobe origin [10]. In addition, the time lag between cardiovascular changes and seizure onset was longer in seizures of temporal lobe origin compared to extratemporal seizures [8, 10]. A study investigating HR changes during epileptic seizures not associated with any subjective or clinical manifestations disclosed tachycardia in eight out of 13 seizures arising from the temporal lobe, but only in one out of nine extratemporal seizures [11].

Therefore our data reflect the findings of previous investigations in which tachycardia was uncommon before clinical seizure onset or in the absence of clinical manifestations in seizures of extratemporal origin. However, the seizure onset zone in our population was identified in the cingulate gyrus only in patient 8 who underwent evaluation with deep implanted electrodes. The pattern of HR changes in this patient were consistent with the group analysis both in the preSP and in the SP, with significant tachycardia observed after seizure onset. In the other seven patients an extratemporal origin of the seizures could only be supposed, as NFLE seizures, particularly with hyperkinetic features, could also originate, albeit less frequently, in the temporal region [12].

Regardless of the explanation for our findings, HR changes did not result in NFLE seizures, a reliable diagnostic marker of seizure occurrence, however it could suggest an extatemporal origin of the seizure.

As the HR changes coincided with motor seizure onset, we were unable to demonstrate if they are the direct consequence of epileptic discharges involving regions of the CAN or a simple reaction to motor manifestations of seizures. In patient 6, a clear epileptic discharge involving the left centro-fronto-temporal derivations was recorded during which tachycardia was observed despite a few movements. This pattern was present in all three seizures analyzed in this patient, but the discharge was observed more than two minutes after seizure onset and therefore changes in HR could be influenced by persisting HR modifications due to the previous clinical behavior.

To better understand the autonomic activation related to NFLE seizures and confirmed by HR analysis, we performed a time and frequency domain analysis of the HRV using wavelet transform technique. As previously explained, this technique detects the sudden shift in sympathovagal balance during transient phenomena and has been applied to describe autonomic changes associated with non epileptic motor phenomena of sleep [13].

Spectral analysis of HRV in our patients showed a significant increase in the LF component that started before clinical seizure onset and persisted throughout the seizure. A significant decrease in the HF component that mainly represents parasympathetic outflow and respiratory rhythm, was instead observed only after clinical seizure onset, as the HF component remained unchanged in the preseizure period. This finding demonstrated that a clear autonomic activation represented by a shift of sympathovagal balance towards sympathetic hypertonus preceded and was therefore independent of the epileptic movement onset. However lack of information on the temporal evolution of the epileptic discharge meant that we can only speculate on the nature of this autonomic activation.

A previous study explored the temporal relationship between autonomic modifications and cerebral and muscular activity related to non epileptic motor phenomena of sleep (periodic limb movements of sleep) using wavelet analysis of the HRV and EEG spectral component. An early sympathetic activation was observed nearly six seconds prior to movement onset and four seconds before an increase in EEG delta activity became evident [13]. The same temporal relationship between cardiac changes, cerebral delta synchronization and PLMS onset was observed in other studies, suggesting a permissive function of the autonomic and cerebral activation on movement occurrence [14, 15]. A continuous spectrum of EEG changes associated with various degrees of autonomic activation characterized also the spontaneous arousal from sleep [16, 17]. The stereotyped pattern of cerebral and autonomic variation preceding PLM is therefore likely to reflect

physiological fluctuations in arousal levels. According to these findings and with the proven association between both major and minor motor episodes of NFLE with phase A of the CAP [18], we could argue that the sympathetic activation observed in our study before seizure onset is the autonomic expression of a spontaneous arousal from sleep that triggers seizure occurrence. How arousal exerts this trigger role, however, remains unsettled. As the low-frequency component of CAP seemed to be localized mostly over the frontal areas [19], an epileptogenic focus localized in the frontal lobe could be more readily activated by this oscillatory mechanism [6]. From this point of view the pathophysiological mechanism underlying NFLE could be primarily related to a dysfunction in the arousal system. The neuronal nicotinic acetylcholine receptor, whose subunits resulted mutated in some families with ADNFLE, has a modulatory effect on the arousal system at both thalamic and cortical levels. The regional distribution and density of this receptor differed in patients with the mutated subunits, and gain of function has been demonstrated [20]. Due to this finding the possible contribution of α4/β2 subunit mutations in the genesis of pathological thalamocortical oscillations triggering epileptic seizures has been suggested [20]. However, studies with intracerebral recordings demonstrated that an increase in the arousal fluctuation observed in NFLE patients is the consequence and not the trigger of epileptic discharges not detectable on scalp EEG. Moreover, a reduced CAP rate and PLM were observed after surgical treatment of NFLE [6, 21]. Taken together these findings, suggest that the autonomic activation observed before seizure onset is probably directly caused or triggered by the epileptic discharge.

A final consideration on our data is that both the HR variations and the pattern of autonomic activation, characterized by an increase in sympathetic activity, were confirmed in all eight patients analyzed during both the preSP and the SP, irrespective of the sleep phase of seizure occurrence or the clinical seizure features. The pattern of HF variations

was more variable, probably due to a biphasic development of changes during the SP as explained for patient 2, or to difference in respiratory activity. One limitation of our study is the difficulty determining changes in respiratory activity by strain-gauge during the SP due to movement artifacts. However, HF pattern differences did not influence the direction of sympathovagal balance changes toward a sympathetic activation in the preSP period and in much of the SP as demonstrated for patient 2. Moreover, visual inspection showed a quite stereotyped pattern of evolution of autonomic activation during different seizures of the same patient (see graphics in appendix A) confirming that stereotypy of clinical manifestations is a feature of NFLE seizures [22]. Despite this stereotypy, as sympathetic activation was also observed before and during non epileptic motor phenomena of sleep and studies applying similar analysis methods to evaluate the evolution of autonomic changes during NREM parasomnias are lacking, we could not asses the diagnostic value of our findings.

5.2 NOCTURNAL ICTAL ASYSTOLE

Bradycardia and asystole are rare epileptic manifestations primarily seen during seizures arising or involving the temporal lobe [23], while cases related to frontal lobe seizures have occasionally been reported [24,25].

The clinical features of our patient with IA were consistent with NFLE (nocturnal and high frequency occurrence of the seizures, seizure motor behavior resembling NPD, poor informative ictal EEG recordings). However compared with our other eight patients, analysis of cardiovascular changes in this patient also yielded different results outside the seizure period.

Firstly a significant tachycardia starting at least 20 seconds before seizure onset was detected in all three seizures analyzed. This early HR modification was associated with changes in EEG activity and an increased respiratory rate, in the absence of any other behaviors. As a clear epileptic discharge was not recorded we could not establish the epileptic nature of these early signs, but this autonomic and electrophysiological activation recurred in a stereotyped fashion even with similar time duration (25, 20, 19 s respectively) in the three seizures evaluated.

The finding of early tachycardia, mainly observed during seizures involving the temporal regions [26], and ictal bradycadia and asystole [23] suggest that the seizure onset zone in our patient could be localized in the mesial areas of the temporal lobe or in the insular cortex. Brain MRI abnormalities are consistent with this hypothesis.

The pattern of HR changes after seizure onset, consisting of tachycardia evolving into progressive bradycardia leading to asystole, was in agreement with previous descriptions

[23, 27]. These HR changes were associated with a marked sympathetic activation before seizure onset and in the first part of the seizure (increase in LF absolute values and LF %). The progressive decrease of HR in the second part of the seizure was associated with a sudden and significant increase in parasympathetic activity and a further increase in LF absolute values. This gradual pattern of HR decrease, associated with LF increase, could be explained by the sympathetic system's attempt to counteract the parasympathetic hypertonus, to prevent asystole. We could argue that finally asystole occurred when the sympathetic exhaustion led the parasympathetic system to prevail.

A paroxysmal discharge was not documented before bradycardia and asystole in our patient and we assume the involvement of the left hemisphere only on the basis of the concomitant clinical behavior characterized by an asymmetric tonic posturing with extension of the right arm and leg, and the brain MRI findings. This hypothesis is in line with results of cortical intraoperative stimulations in humans suggesting a lateralization of parasympathetic activity to the left insular cortex and sympathetic activity to the right [28], and with literature cases which implicated the left hemisphere in the genesis of IB on the basis of ictal and interictal electroencephalographic findings [23].

5.3. CONCLUSIONS AND FUTURE PERSPECTIVES

The present study provides the first description of time-related variations in HR and sympathetic-parasympathetic balance associated with NFLE seizures and nocturnal ictal asystole. Although we can only speculate on the pathophysiological mechanism underlying these centrally mediated events, important conclusions can still be drawn.

Firstly, changes in autonomic balance toward a sympathetic prevalence always preceded clinical seizure onset in NFLE, even when HR changes were not yet evident. Further investigation could focus on correlating the degree of sympathetic activations and seizure occurrence to search for autonomic indicators of impending seizures.

Secondarily, wavelet analysis is a sensitive technique to detect sudden variations of autonomic balance, and could be applied to explore autonomic variations during non epileptic motor phenomena of sleep like NREM parasomnias for a differential diagnosis with seizures.

Lastly, epileptic asystole is associated with a parasympathetic hypertonus counteracted until the end by a marked sympathetic activation. If this pattern were confirmed in other seizures with IA accompanied by a clear epileptic discharge, important information on hemispheric lateralization in cardiovascular control could be gained.

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