

## Brain oscillatory activity during sleep shows unknown dysfunctions in early encephalopathy

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**Abstract** Electroencephalographic recordings in cirrhotic patients without overt hepatic encephalopathy (HE) have mainly been performed during wakefulness. Our aim was to quantify their alterations in nocturnal sleep electroencephalogram (EEG). In 20 patients and 20 healthy volunteers, we recorded a nocturnal digital polysomnography. Different sleep parameters were measured. Besides, we performed quantitative analysis of EEG (qEEG) as follows: spectral power in the different sleep stages was calculated in the frequency bands low  $\delta$ ,  $\delta$ ,  $\theta$ ,  $\alpha$ , and  $\sigma$ . Also, the mean dominant frequency and Sleep Indexes were obtained. In comparison with controls, the group of patients showed (1) different alterations in both the microstructure and the macrostructure of sleep; (2) an increase in, both,  $\theta$  band power and the average mean dominant frequency during rapid

eye movement (REM); (3) in all sleep stages, a decrease of sleep electroencephalogram spectral power in low  $\delta$  band and an increase in  $\delta$  band; and (4) in stages N3 and REM, significant increases in the minimum of mean dominant frequency and in the respective sleep indexes. Therefore, in cirrhotic patients without overt HE, and likely having minimal hepatic encephalopathy, we found different alterations in both the microstructure and the macrostructure of nocturnal sleep. Also, sleep qEEG showed a brain dysfunction in slow oscillatory mechanisms intrinsic of sleep stages, with an increase in the frequency of its maximal electroencephalogram synchronization, from low  $\delta$  to  $\delta$  band. These alterations may reflect the onset of encephalopathy; sleep qEEG may, thus, be an adequate tool for its brain functional evaluation and follow-up.

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## Introduction

One of the major complications that can develop in patients with liver cirrhosis is hepatic encephalopathy (HE); this neurocognitive dysfunction is characterized by its evolution as a continuum from minimal hepatic encephalopathy (MHE) to overt HE [9]. Approximately 30–45 % of cirrhotic patients present overt HE, whereas some 22–80 % of cases with cirrhosis, depending on performed diagnostics, have MHE [4, 7, 16]. This mildest form of HE shows a normal clinical examination, although patients have subtle motor changes and altered sleep, together with cognitive deficits that can only be measured by neuropsychometric testing; this condition impairs health-related quality of life [4, 7, 11, 19, 25].

Electroencephalographic recording in cirrhotic patients has mainly been performed during wakefulness. In the awake state, when there is no overt HE, their electroencephalogram (EEG) shows a slowing that increases with the progression of encephalopathy, with an increase in spectral power in the  $\theta$  band at the beginning, and then in the  $\delta$  band, at more advanced stages. Frequency changes have been characterized by a decrease of the mean dominant frequency (MDF) when compared to control subjects [3, 27, 31], and spectral alterations were correlated with hepatic function indexes. The results of the quantitative analysis of awake EEG (qEEG) in these patients have prognostic value for the appearance of HE bouts and mortality [4, 28]. It can be important to detect and quantify all cerebral alterations associated to the problems present in MHE, since they decrease the quality of life of patients, and probably they deserve to be treated.

So far, there are some studies of sleep disorders in cirrhotic patients with different degrees of EH [10, 14, 22–24], but only Martino et al. [22] focused on the analysis of nocturnal sleep EEG alterations in cirrhotic patients without overt HE.

Moreover, sleep is a physiological state divided into several stages (non-rapid eye movement (NREM) and rapid eye movement (REM)) according to the EEG. NREM sleep (stages N1–N3) is characterized by the presence in the electroencephalogram of low-

frequency rhythms ( $\leq 7$  Hz) [12]. The low-frequency rhythms (1–7 Hz), as pointed out earlier, are altered in cirrhotic patients during wakefulness.

The aim of this study was to determine in cirrhotic patients without overt HE, and likely having MHE, new disturbances in their brain electrical activity and to investigate by qEEG analysis the possible spectral EEG alterations during the different sleep stages. These were, in fact, obtained, as will be later detailed.

## Methods

### Subjects

This study was performed on the recordings made, in 2001, in 20 patients and 20 controls; included in the paper by Martino et al. [22]. The 20 cirrhotic patients (between 41 and 72 years) included 15 men and 5 women without overt HE. This was determined by a neurological exam, checking that they were fully alert; oriented in person, environment, and time; and did not show “flapping tremor” or neurological focal findings. Twenty healthy volunteers matched by age (10 males and 10 females) were also studied.

Patients included in the study had different types of cirrhosis (Table 1). The diagnosis of cirrhosis was confirmed by biopsy, and its severity was characterized according to the classification of Child-Pugh.

Both groups underwent, immediately after polysomnography, a blood sample extraction in order

**Table 1** Demographic and clinical data

	Control <i>N</i> =20	Cirrhotic <i>N</i> =20
Age, in years (SD)	56 (9.02)	57.5 (8.6)
Sex (F/M)	5:15	10:10
Etiology of cirrhosis		7 Virus C 3 Virus B 5 Alcohol 4 Virus C+alcohol 1 Other
Child-Pugh stages		A: 12 B: 5 C: 3

*N* Subject number, *SD* standard deviation, *F* female, *M* male

to obtain the necessary parameters for the classification of Child-Pugh—in patients—and to ensure that these parameters were within normal range in controls; also, an arterial blood sample was taken with the aim to measure ammonia. Psychometric assessment was performed with Number Connection Tests A and B (NCT-A and NCT-B; see Table 2) to assess possible alterations of mental function.

Exclusion criteria were diagnosed sleep disorders; suffering from neurological or psychiatric disease, as well as taking psychoactive medication, such as antidepressants or sedatives, and diuretics; a history of alcohol abuse in the 2 months preceding the study; gastrointestinal bleeding or infectious disease in the month earlier; chronic renal failure, chronic obstructive pulmonary disease; hepatopulmonary syndrome; and massive ascitis with ventilatory alterations.

Controls were in good health. Regarding sleep habits, a detailed clinical history was taken in all controls with the aim of locating and discarding subjects with disturbances during sleep. Subjects with very irregular sleep schedules were also excluded. It was checked with particular attention that they did not suffer, nor have suffered in the past, liver problems, psychiatric illnesses or illnesses that could affect the central nervous system, and that they were not alcohol consumers. Subjects taking psychoactive substances at the time of the study, or taking chronically any medication, were excluded.

The Ethics Committee at our Institution (Comité Ético de Investigación Clínica del Hospital Ramón y Cajal) approved this study and informed consent, which was signed by all subjects before their participation in the study. This included recording sleep EEG with only eight electrodes, electrooculography (EOG) with two electrodes, and electromyography (EMG) with two chin electrodes.

## Recording and signal acquisition

In all subjects, nocturnal digital polysomnography (EEG, EOG, and EMG) was performed during 8 h (from 11 p.m. to 7 a.m.) in a silent room at  $20 \pm 1$  °C. The EEG was recorded with surface electrodes placed according to the International 10-20 System using four bipolar derivations ( $C_3-O_1$ ,  $O_1-T_3$ ,  $C_4-O_2$ , and  $O_2-T_4$ ) and a central monopolar derivation ( $C_Z$ -Mastoid). All electrode impedances were lower than 5 k $\Omega$ . The EOG and the submental EMG were bipolarly recorded, with electrodes placed in the external angle of each orbit, and on the chin, respectively. After 45 Hz low-pass filtering (CyberAmp 380, 24 dB/octave), all signals were digitized at 128 Hz using a 16-bit A/D converter and stored on the PC hard drive. The high-pass filter was set at 0.1 Hz (CyberAmp 380). Before lights were off, awake EEG of the alert subjects was recorded in eyes-open and eyes-closed conditions.

## Processing the recorded signals

Sleep staging was carefully performed according to the criteria of Rechtschaffen and Kales [26]. Artifacts were manually suppressed. In fact, labeling of EEG epochs with obvious artifacts was performed during visual inspection of the polysomnogram for staging by an experienced clinical neurophysiologist blinded to the subject status. These epochs were not used for EEG processing, but they were not erased. Also, the above-cited detection of artifacts was further checked by simultaneous revision of the EEG and the corresponding graphed temporal evolution of both EEG spectral power in the studied frequency bands and the MDF (see later). These did eventually alert on artifacts that, inadvertently, had not been labeled as such during the first visual inspection. In this study, the analyzed sleep stages were N2, N3 (SWS),

**Table 2** Psychometric test and ammonia level alterations in cirrhotic patients

Variables	Control	Cirrhotic	Significance ( <i>p</i> )
NCT-A (s)	<i>35.84 ± 14.77</i>	<i>82.17 ± 44.27</i>	3.05E-5 <sup>a</sup>
NCT-B (s)	<i>81.16 ± 46.02</i>	<i>175.93 ± 86.79</i>	3.35E-5 <sup>a</sup>
Ammonia level (μg/dl)	25.25 ± 14.41	62.95 ± 30.52	0.000

Data in italics correspond to statistical analysis using the nonparametric Mann–Whitney *U* test

NCT-A and NCT-B number connection test

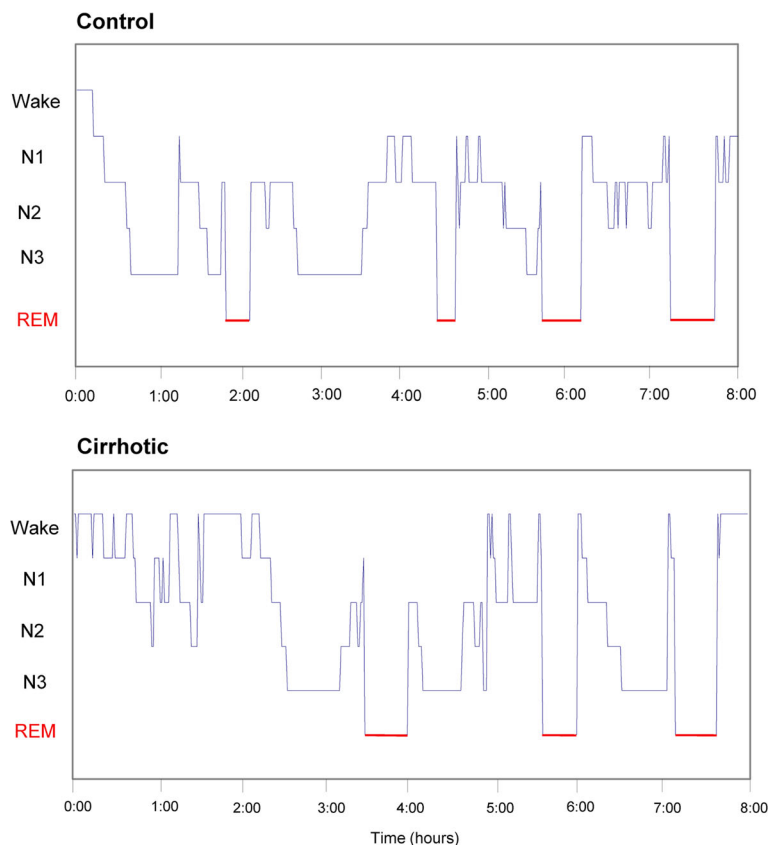
<sup>a</sup> The exact significance for this test; for ammonia level data, Student's *t* test was used. Values are mean ± SD

and REM, according to the new terminology recommended by the American Academy of Sleep Medicine (AASM) [20, 29] although W and N1 were also scored.

### Hypnogram and sleep EEG parameters

Data on the evolution of sleep stages throughout the night were reflected graphically on a hypnogram (see Fig. 1) to have an overall assessment of the quality of sleep. Besides, the following sleep EEG variables based on Rechtschaffen and Kales [26] and named according to the new terminology recommended by AASM [20] were determined (see Table 3), in order to precisely determine the following sleep alterations (architecture and continuity): sleep latency (SL), time spent from lights off to the onset of sleep (first stage N2 in this study), REM latency (RL), wake after sleep onset (WASO), total sleep time (TST), sleep efficiency (SE), number of sleep cycles (SC), time elapsed in the different sleep stages (N2, N3, and R), and time percent in sleep stages (%N2, %N3, and %R).

**Fig. 1** Hypnograms of representative control and cirrhotic subjects. *Upper* control hypnogram shows a normal sleep structure (see text). *Lower* cirrhotic hypnogram shows the characteristic patient sleep alterations as follows: increased latency, lower sleep cycle number, long periods of intra-sleep wakefulness, and altered REM sleep pattern



### EEG spectral characterization

A fast Fourier Transform (FFT) routine was applied to consecutive 1-s epochs weighted by a Hanning window with no overlap. The absolute values were squared to obtain power density values. Then, 60 segments of 1 s were averaged to obtain a power density spectrum in 60-s epochs. These were used, both, in the visual scoring of sleep following the criteria of Rechtschaffen and Kales [25] and for identification of artifacts, as described in the “Processing the recorded signals” section. In each of the 60-s epochs, we calculated the relative power density in the frequency bands low  $\delta$  (0–1 Hz),  $\delta$  (1–4 Hz),  $\theta$  (4–8 Hz),  $\alpha$  (8–12 Hz), and  $\sigma$  (12–15 Hz) with respect to the power in the frequency range from 1 to 45 Hz, and the MDF value. Data were digitally filtered in these bands to visually compare frequency components of EEG tracings on each subject of both groups. The O<sub>1</sub>-T<sub>3</sub> derivation and corresponding spectra are shown in the figures, as the most suitable for studies of HE [3, 31].

**Table 3** Sleep EEG parameters in cirrhotic and control groups; statistical differences

Sleep EEG parameters		Control	Cirrhotic	Significance ( <i>p</i> )
Latency	SL (min)	<i>15.07±16.92</i>	<i>37.03±32.32</i>	<i>0.003*</i>
	RL (min)	128.53±55.67	157.24±90.13	0.233
Wake–sleep	WASO (min)	<i>24.3±19.17</i>	<i>51.2±44.93</i>	<i>0.027*</i>
	TST (min)	<i>412.9±48.8</i>	<i>354.3±75.67</i>	<i>0.011*</i>
	SE	<i>88.85±8.64</i>	<i>76.80±16.07</i>	<i>0.007*</i>
Cycles and sleep stages	SC	<i>3.85±0.93</i>	<i>2.6±1.5</i>	<i>0.005*</i>
	N2 (min)	116.4±55.12	95.85±45.8	0.208
	N3 (min)	185.2±57.4	143.75±61.62	0.034*
	R (min)	83.75±31.57	47.80±33.77	0.001*
	% N2	27.95±11.64	27.15±10.74	0.823
	% N3	45.35±14.18	40.03±15.72	0.268
	% R	19.67±6.1	12.21±8.49	0.003*

Data in italics correspond to statistical analysis using the nonparametric Mann–Whitney *U* test; for all other data, Student's *t* test was used. Values are mean±SD

SL sleep latency; RL REM latency; WASO wake after sleep onset; TST total sleep time; SE sleep efficiency; SC number of sleep cycles; N2, N3, and R time elapsed in the different sleep stages; %N2, %N3, and %R time percent in sleep stages

\**p*<0.05, statistically significant differences

The MDF defined as mean frequency weighted by power, was calculated according to the following formula in the frequency range 0.5–45 Hz:

$$MDF = \frac{\sum_{i=1}^n f_i \cdot s_i}{\sum_{i=1}^n s_i}$$

where  $f_i$ =frequency  $i$ ,  $s_i$ =power at frequency  $i$ , and  $n$ =number of frequencies; 45 in this study.

#### EEG variables in each sleep stage

After processing the complete 8-h recording, the variables used to characterize the EEG in each sleep stage were MDF (average and minimum) and relative spectral power in the frequency bands low  $\delta$  (0–1 Hz),  $\delta$  (1–4 Hz), and  $\theta$  (4–8 Hz). Also, indexes were defined for each phase of sleep to quantify the relation between the EEG spectral power in the frequency bands characteristic of that phase and the slowest frequency band; they were named like the respective phases: N2=( $\delta$ + $\theta$ )/low  $\delta$ , N3= $\delta$ /low  $\delta$ , REM=( $\delta$ + $\theta$ )/low  $\delta$ . These variables were calculated for each of the subjects in each group

(cirrhotic and control) and each of these variables was then averaged in each group; these averages were the values that were used in the statistical analysis.

#### Statistical analysis

First, we performed the Shapiro–Wilk test to verify whether the study variables were following a Gaussian distribution, with a significance level of 0.05. Then, all variables were analyzed to find possible significant differences between groups by means of two types of statistical analyses: Student's *t* test for two independent samples (when the variables had a Gaussian distribution) or the nonparametric Mann–Whitney *U* test for two independent samples (when the variables had not a Gaussian distribution) in both analyses with a significance level of 0.05. In addition, we performed a bivariate correlation analysis between the psychometric test and ammonium levels with EEG variables in all sleep stages. We obtained Pearson's correlation ( $r$ ) or Spearman's correlation coefficient ( $r_s$ ) depending on whether the variables followed a normal distribution or not, and we selected a significance level of 0.05. All analyses were performed with IBM SPSS Statistics version 20.

## Results

The alterations in the macrostructure of sleep in studied cirrhotics (Table 1) are shown by representative hypnograms (Fig. 1); the disorders in psychometric test and ammonia level found in these patients are quantified in Table 2 and the results of statistical analysis differences in sleep EEG parameters in Table 3. Figures 2, 3, and 4, show an example of the differences between a control subject and a patient in the raw and digitally filtered recordings of sleep EEG, as well as their power spectra and MDF, in the different sleep stages. The spectral EEG characterization shown in these figures and the results of statistical analysis shown in Figs. 5 and 6 and Table 4 reveal the alterations in the microstructure of sleep in cirrhotic subjects.

### Demographic, psychometric, and clinical data

Demographic, clinical, and psychometrics data as well as statistical significance of the differences between the two groups are shown in Tables 1 and 2. According to Shapiro–Wilk test results, age, and ammonia level followed normality criteria whereas psychometric tests NCT-A and NCT-B did not. Mean comparison analysis using Student's *t* test for age and ammonia level, did not find any statistically significant differences between patient and control groups for age; nevertheless, patients showed an increased ammonia level that was statistically significant with respect to the control group (Table 2). On the other hand, the nonparametric Mann–Whitney *U* test used to analyze differences between NCT-A and NCT-B showed a statistically significant increased time of performing the test in patients (Table 2).

### Wakefulness EEG

The EEG recorded during wakefulness in cirrhotics showed the characteristic slowing described for these patients in the literature [3] (not shown). As mentioned above, the aim of this study focused on the analysis of nocturnal sleep EEG.

### Sleep EEG: hypnogram and sleep EEG parameters

In the hypnogram examples shown in Fig. 1—belonging to the control subject and the cirrhotic patient whose EEG raw data and processing will be shown next—the following is noteworthy: the control subject hypnogram

(Fig. 1, upper) shows rapid sleep onset, normal sleep structure with four cycles, few awakenings during the night, and adequate REM sleep profile. The cirrhotic hypnogram (Fig. 1, lower) shows that the patient sleep latency is increased with an unstructured sleep, only three sleep cycles, long periods of intrasleep wakefulness, and altered REM sleep pattern.

In order to study differences in sleep parameters, we performed mean comparison analysis using Student's *t* test in the following parameters RL, time in N2, N3, and R, and %N2, %N3, and %R because these parameters followed a Gaussian distribution, and we used nonparametric Mann–Whitney *U* test for SL, WASO, TST, SE, and SC because they did not follow a normal distribution. Results of these analyses showed the presence of statistically significant differences between groups in SL, WASO, TST, SE, SC, N3, R, and %R (see Table 3).

From the above results, we can summarize that the most striking results of the macrostructure of sleep study in cirrhotics are: impaired sleep onset, altered sleep maintenance, increased time awake during the night, and decreased sleep efficiency (Fig. 1 and Table 3). They also include a change in the duration of sleep stages in cirrhotics, with significantly less time of REM and N3 stages, and a nonsignificant reduction in N2 stage. Also, a lower number of complete sleep cycles is depicted.

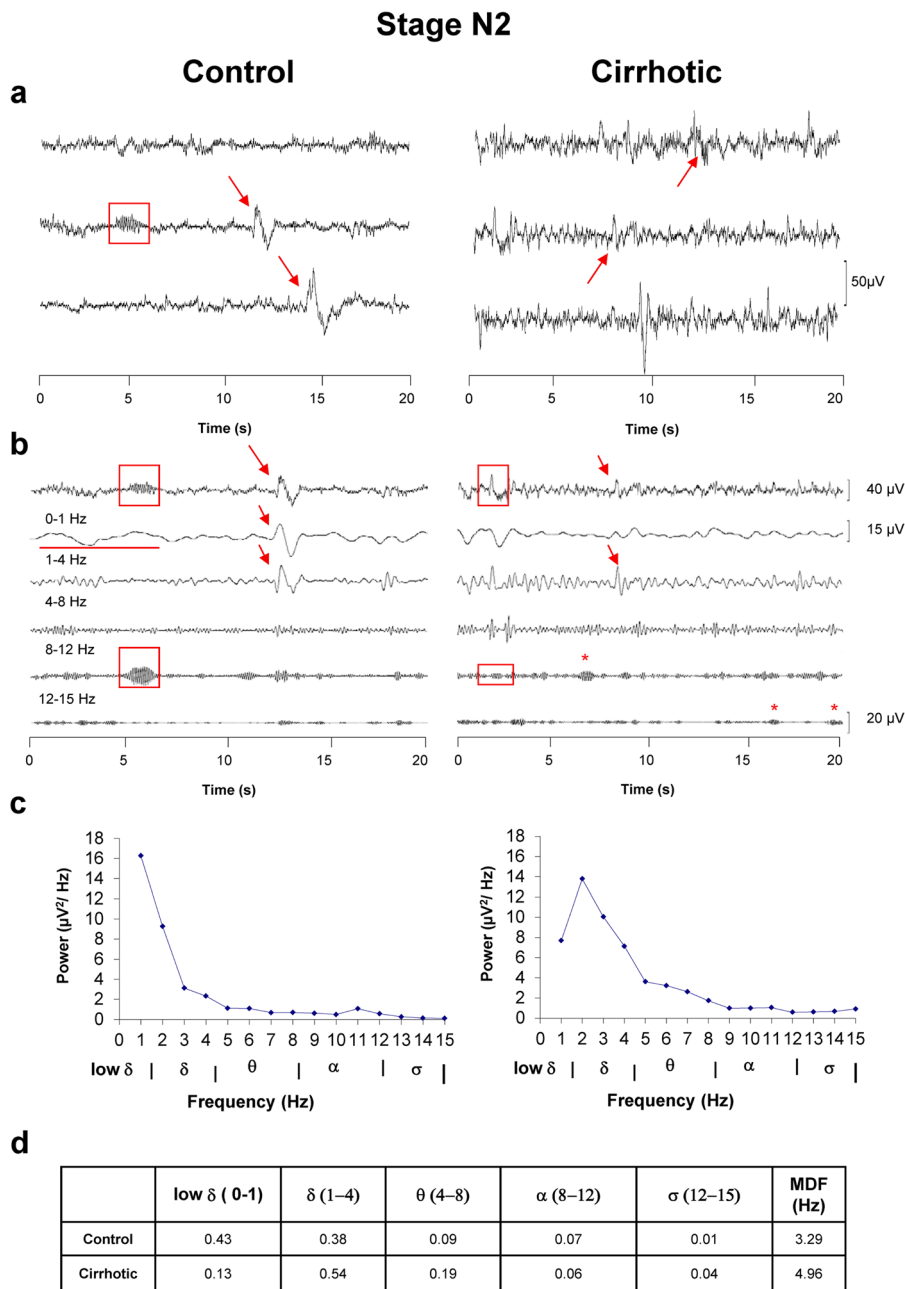
### Sleep stages EEG

#### *EEG digital filtering and spectral characterization*

The electroencephalographic signal of patients recorded during nocturnal sleep showed clear graphoelement and spectral differences with respect to controls in all sleep stages. This is illustrated by showing example of a control subject and patients in each of the stages of sleep as follows:

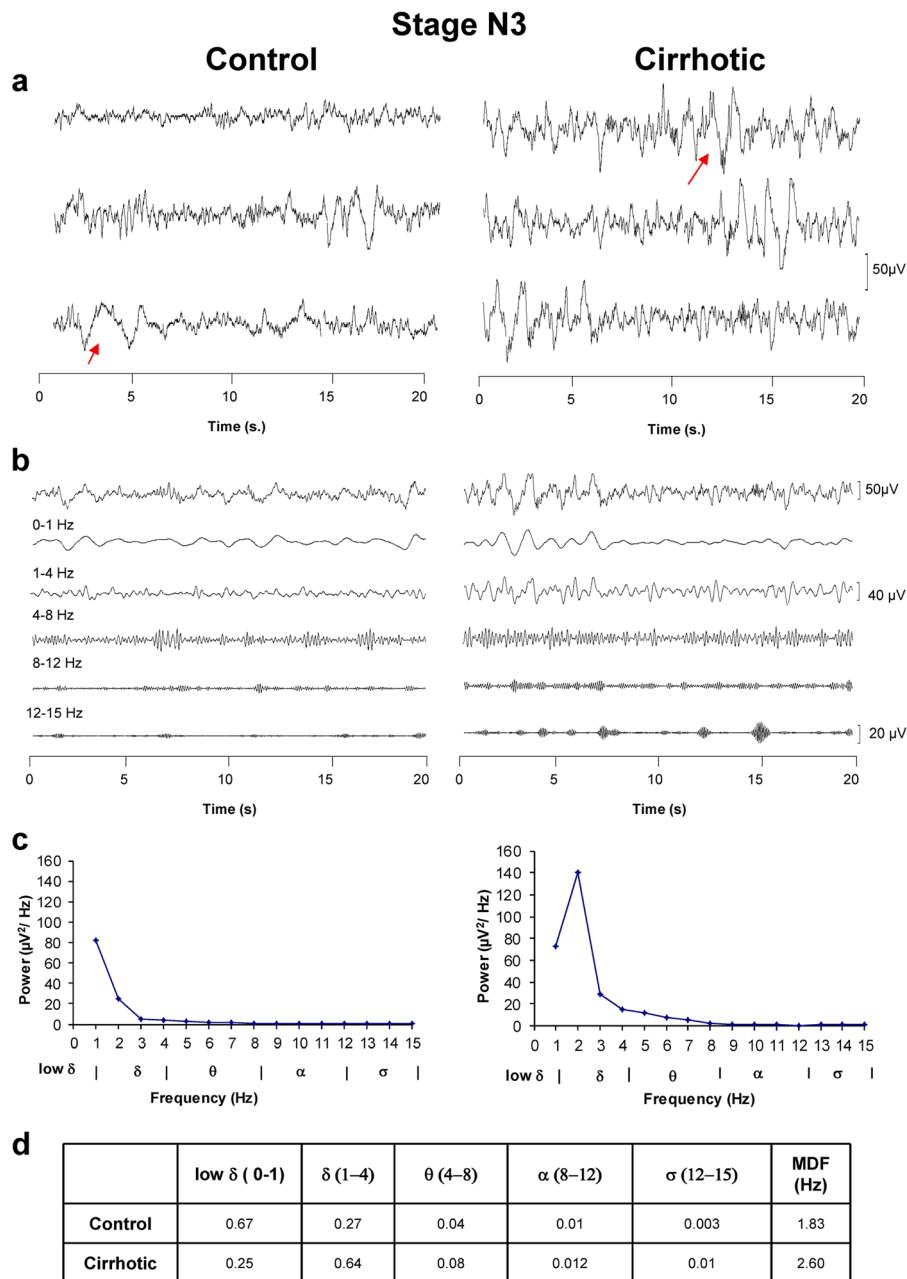
In stage N2, the cirrhotic EEG ongoing activity shows a larger, more synchronized activity (Fig. 2a, right) than the control one. K complexes and spindles are less evident in the cirrhotic. In the digitally filtered records (Fig. 2b, right) it can be seen that the cirrhotic tracing from 0 to 1 Hz (low  $\delta$  band) has lower amplitude in the slow component of K complex than the control one, although this does not, by itself, imply a lower delta power in stage N2, since the latter is not exclusively accounted for by K complexes. Also, from 1 to





**Fig. 2** Sleep stage N2 EEG is different in representative examples of control and cirrhotic subjects; digitally filtered and spectral data. **a** Raw EEG records. Differences between characteristic cirrhotic (*right*) and control subjects (*left*) are noticeable—sleep spindles (*rectangle*) and K complexes (*arrows*). The three 20-s tracings are consecutive. **b** Filtered records. Upper recording, 20 s of raw EEG, from data shown in **a**; other tracings, same data but digitally filtered in the indicated frequency bands. *Left* control, *rectangles* show sleep spindles. In the tracing filtered between 0–1 Hz (low  $\delta$  band), a *horizontal line* indicates the slowest oscillation present. *Right* cirrhotic, the *rectangle* in the upper tracing indicates a K complex; *arrows* indicate another K complex. **c** Power spectra,

obtained with the 60 s of EEG presented in **a**, show that in the cirrhotic (*right*), there is a decrease in absolute power at frequencies <1 Hz, accompanied by a substantial increase at frequencies within the  $\delta$  band. Frequency bands are indicated below the abscissa axis. **d** Table with relative spectral power of example EEGs presented in **a** in the indicated frequency bands and the MDF (*right column*, in Hertz) of the above power spectra. *First row* control, *second row* cirrhotic. As will be seen in Fig. 5 (*upper left*) for significant group differences in cirrhotic relative power in N2, it decreased in low delta, and increased in delta and theta bands



**Fig. 3** Sleep stage N3 EEG is different in representative examples of control and cirrhotic subjects; slowest epoch in the night. Samples taken at time when MDF reaches the respective minimum values of the whole night in both subjects. **a**, **b**, **c**, and **d**, as in Fig. 2. **a** In the consecutive 20-s tracings, the cirrhotic shows slow waves which are more abundant, and of larger amplitude and shorter duration, than the control. **b** Upper record, from raw data

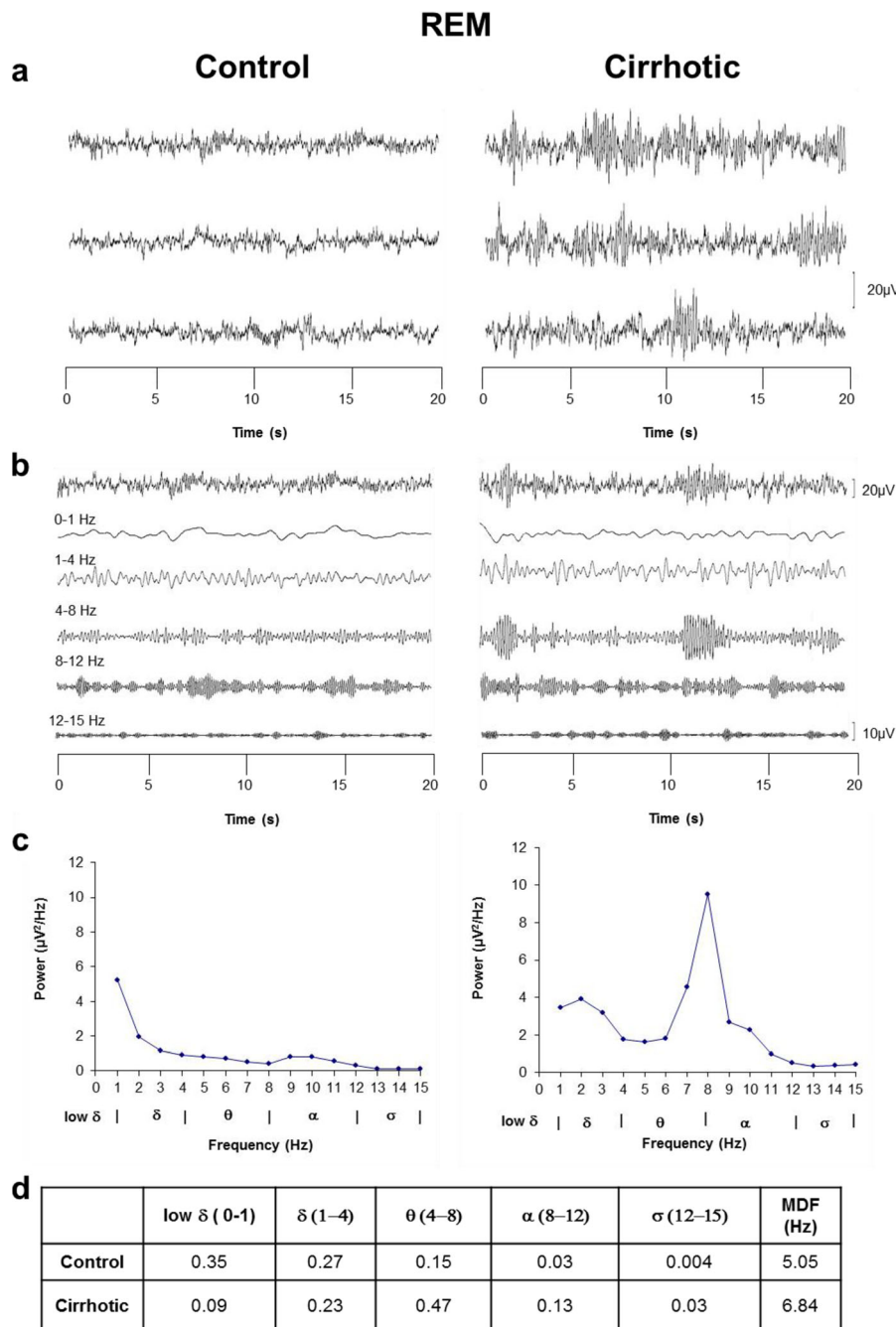
8 Hz (i.e.,  $\delta$  and  $\theta$  frequency bands) waves have higher amplitude than the control ones.

Power spectra of EEG records in Fig. 2c show that in the frequency band below 1 Hz (low  $\delta$ ), the

shown in **a**. Other tracings, digitally filtering of the above show larger amplitudes in the cirrhotic in the bands from 1 to 8 Hz. **c** The power spectrum on the *right* reflects the latter cirrhotic changes. **d** As will be seen in Fig. 5 (*center left*) for significant group differences in relative power in N3, low delta power decreased, and only delta power increased

cirrhotic absolute power value is less than 50 % the control one (7.69 vs. 16.27; Fig. 2c), whereas absolute power in bands  $\delta$  and  $\theta$  is two and three times larger, respectively, than in control (30.97 vs. 14.71





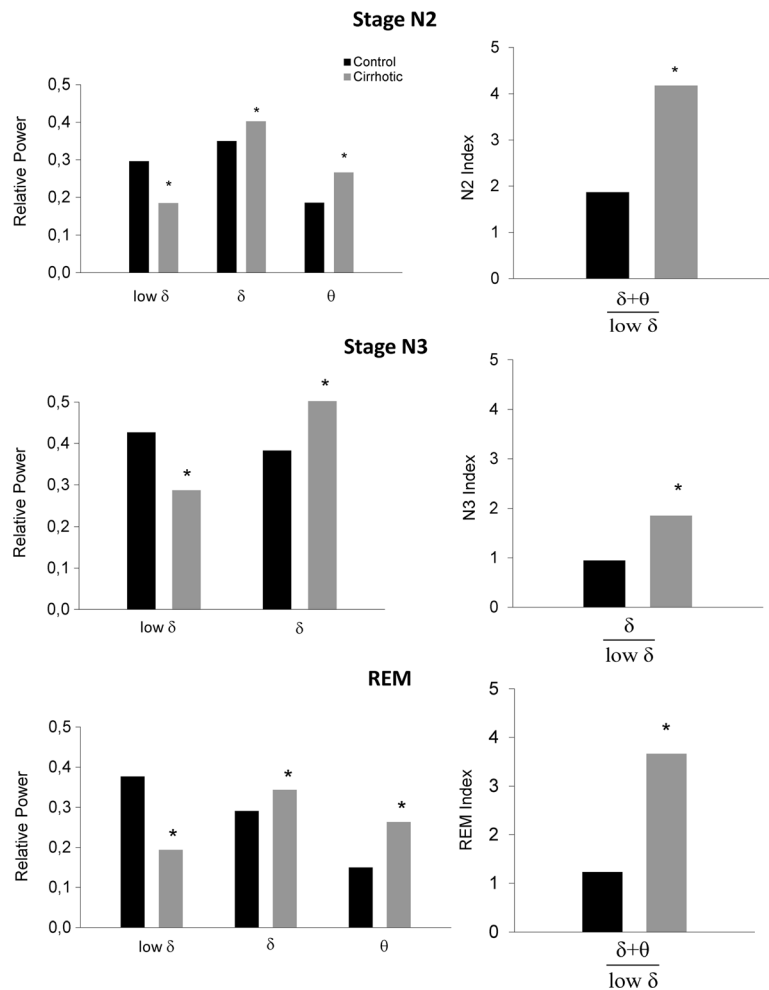
**Fig. 4** REM sleep stage EEG examples show larger theta activity in a representative example of cirrhotics compared to a representative example of controls. **a**, **b**, **c** and **d** as in Figs. 2 and 3. Notice in **a** that cirrhotic EEG (*right*) shows a larger amplitude theta activity than the control one (*left*). **b** Upper record, 20 s of raw data shown in **a**. Notice in **b** (*right*) the larger amplitude in the

cirrhotic filtered theta band (4 to 8 Hz) characterized by the conspicuous theta peak shown in the power spectrum in **c** (*right*). As will be seen in Fig. 5 (*lower left*) for significant group differences in relative power in REM, low delta decreased, and delta and theta increased

and 10.89 vs. 3.41; Fig. 2c). The corresponding values in cirrhotic relative power (Fig. 2d) are, in the frequency band below 1 Hz around 30 % the

control one (0.13 vs. 0.43; Fig 2d), whereas relative power in band δ is around 40 % larger than the control one and around 100 % larger in band θ

**Fig. 5** EEG spectral power and sleep indexes show significant changes in cirrhotics. Spectral bands relative power (*left*) and sleep indexes (*right*), in control and cirrhotic groups, showed differences in the three sleep stages (*upper to lower rows*). *Left* histograms of mean relative spectral power, *right* sleep indexes at each sleep stage. In this and the following figure, *asterisks* indicate statistically significant differences between both groups ( $p < 0.05$ ; for details, see Table 4). Data are mean values from all subjects in each group



(0.54 vs. 0.38 and 0.19 vs. 0.09, respectively; Fig. 2d) than in control. The two latter are reflected in the increase of MDF from 3.29 to 4.96 Hz (Fig. 2d).

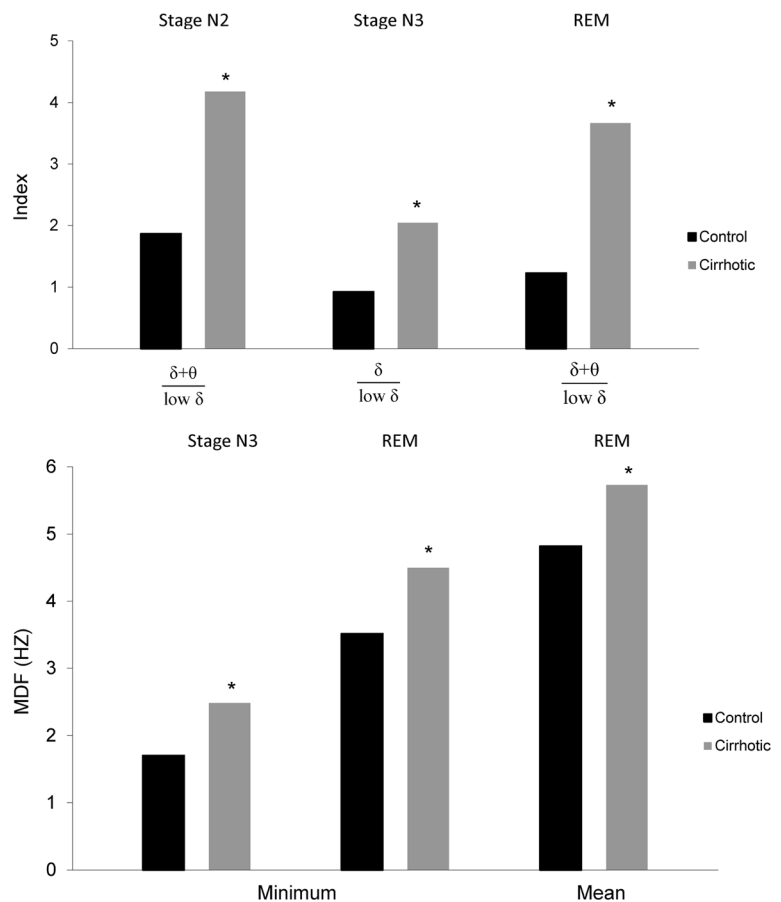
In stage N3, often called slow-wave sleep, the cirrhotic EEG also changed the frequency of its maximal synchronization, from low  $\delta$  to  $\delta$  band (Fig. 3a, b, 0–1 and 1–4 Hz), a change that was quantified by the power spectrum (see peak in Fig. 3c, right). This is also noticeable in the filtered data (Fig. 3b, row 1–4 Hz), since there is an increase in slow wave amplitude in the cirrhotic with respect to the control. In the raw data, these slow waves show a slight frequency increase with respect to the control ones, and are therefore seen both in the filtered records corresponding to 0–1 and 1–4 Hz in the cirrhotic, but only in the former one in the control. This is also reflected in the increase of cirrhotic MDF (Fig. 3d).

In REM, the cirrhotic EEG also showed a very noticeable change with respect to the control one, i.e., there are conspicuous bursts of activity at 8 Hz (Fig. 4a, b), that originated an important increase of spectral power in the 4–8 Hz band with respect to the control one (Fig. 4c). This is also reflected in the increase of MDF in the theta band (Fig. 4d), even though there was a decrease in cirrhotic power in the low delta band, with respect to the control one.

#### EEG differences between cirrhotic and control groups

According to the results of the Shapiro–Wilk test, we used the Student's  $t$  test in the following variables; N2 stage—N2 Index, low  $\delta$ ,  $\delta$ , and  $\theta$ ; N3 stage—MDF minimum, low  $\delta$  and  $\delta$ ; and REM—MDF minimum, MDF mean, REM Index,  $\delta$ , and  $\theta$ . Conversely, we used

**Fig. 6** Sleep Indexes and mean dominant frequency (MDF) changes in early hepatic encephalopathy. *Upper* EEG Indexes were defined to characterize, in the three stages of sleep, the ratios of significant spectral power changes in the frequency bands histograms shown in Fig. 5 (left). *Lower* (in the left and center pairs of the bars) the minimum MDF reached by the EEG in each sleep stage showed significant increases in stages N3, and REM in cirrhotics compared with the control group. Likewise, the mean value of the MDF calculated in each sleep stage was significantly larger during REM in cirrhotics (right bars)



the nonparametric Mann–Whitney  $U$  test in the rest of variables; N2 stage, MDF mean and MDF minimum; N3 stage, N3 Index, MDF mean, and  $\theta$ ; and REM, low  $\delta$ . Results of these analyses about the EEG signal of cirrhotic patients compared to controls at each stage of sleep, presents an increase in the activity above 1 Hz, both in the raw signal (see examples in Figs. 2, 3, and 4) and in the corresponding power spectra.

These differences were evident between the two groups (cirrhotic and control) through statistical analysis, both in the aspect of the distribution of relative spectral power and in the frequency of the recorded signals, as evidenced by the results shown in Figs. 5 and 6 and in Table 4.

In all stages of sleep (Fig. 5), the group of patients showed in comparison to the control group, a decrease in relative power in the low  $\delta$  frequency band and an increase in the  $\delta$  frequency band; both changes were statistically significant (Table 4). There was also a statistically significant increase in the  $\theta$  band in stages N2 and REM (Table 4). This different spectral power distribution in each group was expressed by calculating an

Index, which shows, as mentioned earlier, the relationship between power in the frequency bands characteristic of the different stages of sleep and in the slowest frequency band (low  $\delta$ ), in each of the cited stages (Fig. 5 (right) and Fig 6 (upper)), resulting in a statistically significant increase in each of them in the cirrhotic group compared to the control (Table 4).

The differences in terms of EEG frequency between the two groups were evaluated by calculating the mean and minimum values of MDF at each stage of sleep. Differences between the groups were statistically significant increases in the patient group compared to the control group, both in the minimum value of the MDF at stages N3 and REM and in the average value of MDF in the REM stage of sleep (Fig. 6 (lower) and Table 4).

Correlations between psychometric tests and ammonia with EEG variables in sleep stages

From the results obtained in the correlation analysis between psychometric tests and ammonia with EEG

**Table 4** EEG variables in sleep stages, in cirrhotic and control groups; statistical differences

Sleep stage EEG variables	Control	Cirrhotic	Significance ( <i>p</i> )
Stage N2 variables			
N2 Index [( $\delta+\theta$ )/low $\delta$ ]	1.86 $\pm$ 0.4	4.18 $\pm$ 1.91	0.000*
MDF mean	4.77 $\pm$ 0.43	5.12 $\pm$ 1.09	0.417
MDF minimum	3.65 $\pm$ 0.49	4.03 $\pm$ 0.95	0.160
low $\delta$ (relative power)	0.29 $\pm$ 0.05	0.18 $\pm$ 0.64	0.000*
$\delta$ (relative power)	0.35 $\pm$ 0.03	0.40 $\pm$ 0.07	0.007*
$\theta$ (relative power)	0.18 $\pm$ 0.04	0.26 $\pm$ 0.07	0.000*
Stage N3 variables			
N3 Index ( $\delta$ /low $\delta$ )	0.92 $\pm$ 0.23	2.04 $\pm$ 1.2	0.000*
MDF mean	3.08 $\pm$ 0.3	3.34 $\pm$ 0.66	0.304
MDF minimum	1.7 $\pm$ 0.26	2.48 $\pm$ 0.41	0.000*
low $\delta$ (relative power)	0.42 $\pm$ 0.06	0.28 $\pm$ 0.08	0.000*
$\delta$ (relative power)	0.38 $\pm$ 0.05	0.50 $\pm$ 0.08	0.000*
$\theta$ (relative power)	0.12 $\pm$ 0.03	0.15 $\pm$ 0.05	0.064
REM variables			
REM Index [( $\delta+\theta$ )/low $\delta$ ]	1.23 $\pm$ 0.37	3.66 $\pm$ 1.57	0.000*
MDF mean	4.82 $\pm$ 0.58	5.72 $\pm$ 1.12	0.003*
MDF minimum	3.51 $\pm$ 0.72	4.49 $\pm$ 1.05	0.002*
low $\delta$ (relative power)	0.37 $\pm$ 0.07	0.19 $\pm$ 0.75	9.51E-8 <sup>a</sup>
$\delta$ (relative power)	0.29 $\pm$ 0.03	0.34 $\pm$ 0.09	0.016*
$\theta$ (relative power)	0.15 $\pm$ 0.04	0.26 $\pm$ 0.08	0.000*

Data in italics correspond to statistical analysis using the non-parametric Mann–Whitney *U* test, for all other data, Student's *t* test was used

<sup>a</sup>The exact significance for this test. Values are mean  $\pm$  SD

\**p*<0.05, statistical significances

variables in sleep stages, we describe only those with, both, statistically significant correlations and with a correlation coefficient >0.4 (Table 5). In all stages of sleep, psychometric tests correlated negatively with the

low  $\delta$  band and positively with the index for each sleep stage and with the relative power in the  $\theta$  band. In the N3 stage, the correlation was positive with  $\delta$  band. In both REM and N3 stages, psychometric tests correlated

**Table 5** Results of correlation analysis between psychometric test, ammonia level, and sleep EEG variables in sleep stages

		Stage N2 variables			Stage N3 variables				Stage REM variables				
		N2 Index	Low $\delta$	$\theta$	MDF minimum	N3 Index	Low $\delta$	$\delta$	$\theta$	MDF minimum	REM Index	Low $\delta$	$\theta$
NCTA	$r_s$	0.581	<b>-0.573</b>	0.443	0.448	0.528	<b>-0.470</b>	0.493	-	0.418	0.664	<b>-0.650</b>	0.590
	Significance ( <i>p</i> )	0.000	<b>0.000</b>	0.006	0.005	0.001	<b>0.003</b>	0.002	-	0.012	0.000	<b>0.000</b>	0.000
NCTB	$r_s$	0.504	<b>-0.504</b>	0.425	-	0.520	<b>-0.483</b>	0.460	-	0.402	0.670	<b>-0.636</b>	0.541
	Significance ( <i>p</i> )	0.003	<b>0.003</b>	0.014	-	0.002	<b>0.004</b>	0.007	-	0.020	0.000	<b>0.000</b>	0.001
Ammonia	$r_s$	-	-	-	-	0.424	-	-	0.438	-	-	-	-
	Significance ( <i>p</i> )	-	-	-	-	0.006	-	-	0.005	-	-	-	-
	$r$	0.530	<b>-0.502</b>	0.568	0.587	-	<b>-0.490</b>	0.414	-	-	0.497	-	0.442
	Significance ( <i>p</i> )	0.000	<b>0.001</b>	0.000	0.000	-	<b>0.001</b>	0.008	-	-	0.001	-	0.005

Only correlations that were statistically significant (*p*<0.05) and with correlation coefficient >0.4 are included. Negative correlations are shown in bold

*r* Pearson's correlation coefficient,  $r_s$  Spearman's correlation coefficient

positively with the minimum of the MDF. With respect to correlations with the levels of ammonia, in all sleep stages, we found a positive correlation with the index for each stage of sleep, and with the  $\theta$  band. In the N2 and N3 stages, the correlation was negative with low  $\delta$ , and, finally, in the N3 stage we found a positive correlation with the minimum of the MDF.

## Discussion

The group of patients showed, as noted above, statistically significant hyperammonemia and increased execution time in psychometric tests compared to the control group, both compatible with the presence of MHE. Although for an accurate diagnosis of MHE, it would be necessary, at present, to carry out some more specific tests—non-available when recordings were made—we can describe our patients as likely having MHE.

Our results on sleep EEG parameters show that cirrhotic patients have a very poor sleep quality, with an altered architecture which reflects clear changes in sleep macrostructure in our patients group, consistent with the previously described [8, 14]. Nevertheless, the main interest of this work focused on the investigation of quantitative analysis of sleep EEG in cirrhotic patients without overt HE, and likely having MHE.

In our study, the analysis of nocturnal sleep EEG in cirrhotic patients quantified new changes in the EEG compared to controls, showing an increased activity above 1 Hz, with higher mean frequencies, in all sleep stages. We suggest that there is an alteration of the various oscillations characteristic of the different stages of sleep. The group of cirrhotics reflected a degree of inability to generate the slowest EEG oscillations (<1 Hz) during sleep. In fact, the minimum MDF reached by the EEG in cirrhotics during stages N3 and REM was significantly larger than in the control group (Fig. 6 (lower) and Table 4). Furthermore, nocturnal sleep qEEG also showed both a prominent  $\theta$  activity in REM, and an increase in the frequency of its maximal EEG synchronization in stages N2 and N3, from low  $\delta$  to  $\delta$  band, characterizing brain dysfunction in slow oscillatory mechanisms during sleep (Figs. 5 and 6). These abnormalities indicate that this apparently asymptomatic phase of HE is in fact not so benign, as there is an involvement which also affects brain mechanisms responsible of normal sleep.

A correlation between indexes of liver function and psychometric performance with EEG spectral analysis has been previously described in the waking condition [4]. The alterations caused by hyperammonemia in brain neurotransmission and cognitive impairment have been described in [2, 11, 17, 18]. Our correlation analysis results show statistically significant correlations of moderate magnitude between execution time in psychometric tests and EEG variables in sleep stages, as well as between ammonia and EEG variables in sleep stages (see Table 5). These results show, first, that, similarly to the waking condition, in all sleep stages, there are correlations between psychometrics test and ammonia with EEG spectral analysis in terms of relative power and frequency, and, second, the possible correlations between sleep EEG spectral alterations with the pathophysiological mechanisms of HE due to hyperammonemia as described above.

According to [30], in NREM, there is an integration of the cortical slowest oscillation (<1 Hz) with the oscillations of 1–4 Hz, both cortical and thalamic. The former, at frequencies <1 Hz, commands the other EEG oscillations, delta, and sleep spindles [6]. These authors propose an involvement of glial-neuronal interaction in the <1 Hz oscillation. We found a statistically significant negative correlation between the level of ammonia and the relative power in the low  $\delta$  band, in N2 and N3 sleep stages (Table 5). This finding is consistent with a possible correlation between the decrement in the generation of the EEG oscillations <1 Hz during sleep and the alterations on the pathophysiological mechanisms of HE due to hyperammonemia described in [2, 17]. These studies describe an altered glutamatergic synaptic regulation, with involvement of NMDA receptors and type II astrocytes. Furthermore, ammonium modifies neuronal and glial properties, thereby interfering the generation of <1 Hz oscillations [1, 21].

Moreover, it is noteworthy that while this low  $\delta$  oscillation is only generated during slow wave sleep (stage N3) [6] in cirrhotic patients, the decrease of the EEG power at frequency <1 Hz occurs in all phases of sleep and the minimum of the MDF is increased only in stage N3 and REM. The physiological significance of the alteration in REM sleep remains, so far, unknown.

According to [5, 6, 15], low  $\delta$  oscillation would direct and organize both cortical and thalamic  $\delta$  oscillation. Our patients not only have an alteration in the low  $\delta$  oscillation, but also in  $\delta$  oscillation, evidenced by the fact that the relative power of EEG in the  $\delta$  band

(1–4 Hz) is higher in the group of patients than in the control group at all stages of sleep (Fig. 5, left). These changes are also reflected in the defined Sleep Indexes (Fig. 6, upper). Interestingly, these indexes, significantly larger in cirrhotics in all sleep stages, could be a way to detect early stages of HE.

We ignore whether the change in the  $\delta$  oscillation (1–4 Hz) found in our patients corresponds to an alteration in the cortical or thalamic oscillation, or both, or even if it is caused by the low  $\delta$  disturbance. If we bear in mind that the reflection of the intrinsic thalamic activity in the recorded EEG is questionable, specially without an external synchronization component [5] and the fact that cortical neurons have an intrinsic capability of firing at the  $\delta$  frequency band [13], it may be suggested that the increase in relative power in the  $\delta$  frequency band in the group of patients, may be due to the cortical  $\delta$  oscillation rather than to the thalamic  $\delta$  oscillation, not to mention the possible contribution of K complexes in stages N2 and N3 of sleep. The analysis of changes in the relationships of K complexes with slow waves and of sleep spindles in these patients is been performed at present.

It is noteworthy that in all studied sleep stages, cirrhotics with non-overt HE showed increased EEG delta activity, and increased theta activity except in stage N3; MDF in REM was significantly larger than in controls.

A limitation of this study could be the fact that sleep EEG was recorded with only eight electrodes, as approved by the Ethics Committee at our Institution, since it does not allow the study of the distribution of observed changes. Also, we must emphasize that recordings were made, and staged, in 2002, when Rechtschaffen and Kales criteria—with their limitations—were used. Finally, as the small sample size used recommended a non-parametric test for comparison of our EEG variables, we used the very strict Mann–Whitney *U* test, clearly indicated in this case. Further studies on this subject could confirm the results found in this work.

The conclusions we can draw from these results are, primarily, that cirrhotic patients without overt HE, and likely having MHE, show different alterations in both the microstructure and the macrostructure of sleep; secondarily, the importance of studying the sleep period in cirrhotic patients without overt HE. In fact, the above-studied alterations of EEG during sleep showed an increase in the frequency of its maximal electroencephalogram synchronization, from low  $\delta$  to  $\delta$  band, characterizing a brain dysfunction in slow oscillatory mechanisms during sleep. This may be an early sign of

initiation—and possible progression—of the disease, and can also serve as a control of the evolution and response to applied treatments. It is also noteworthy that the brain electrical activity recorded with electroencephalography during the sleep period may disclose heretofore unknown brain oscillatory dysfunctions in early encephalopathies. Therefore, it may be of interest to include the study of sleep EEG for brain functional evaluation and follow-up in the early phase of other encephalopathies, due to the fact that it completes the study of brain electrical activity, usually performed during wakefulness that does not reveal alterations of some oscillations intrinsic of sleep stages.

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