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Cortical networks are disturbed in people with cirrhosis even in the absence of neuropsychometric impairment

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Potential competing interests

None

HIGHLIGHTS

- Neuronal networks were explored in patients with cirrhosis using advanced EEG computational analysis.
- Significant disruption of cortical networks was found even in the absence of hepatic encephalopathy.
- The findings provide new insights into the cortical signature of this syndrome and its pathogenesis.

ABSTRACT

Objective: Hepatic encephalopathy is a common complication of cirrhosis; it is characterised by neuropsychometric/neurophysiological abnormalities. Its pathophysiology is complex but glial neuronal communication is likely to be disrupted and to impact on oscillatory networks and cortical connectivity. The aim of this study was to use multichannel electroencephalography (EEG) to investigate functional connectivity as a surrogate for cortical networks in patients with cirrhosis.

Methods: Resting EEGs were recorded in 98 healthy controls and in 264 patients with cirrhosis characterised psychometrically using the Psychometric Hepatic Encephalopathy Score (PHES). Functional connectivity was calculated using the phase-lag index with stratification into standard EEG frequency bands. The findings were validated in a further cohort of 39 healthy controls and 106 patients with cirrhosis.

Results: Widespread disruption in functional connectivity was observed in the patients compared with the controls; connectivity was increased in the theta (4-8 Hz) band and decreased in the delta (1–3.5 Hz), alpha (8.5–13 Hz) and beta (13.5–26.5 Hz) bands. Changes were apparent even in patients who were psychometrically unimpaired compared with healthy controls *viz* mean ± SEM theta 0.107±0.001 *vs*. 0.103±0.002 ($p<0.05$) and alpha.0.139±0.003 *vs.* 0.154±0.003 ($p<0.01$); more pronounced changes were observed with increasing neuropsychometric impairment. Findings were replicated in the second cohort.

Conclusion Cortical networks are disturbed in patients with cirrhosis even in the absence of psychometric impairment.

Significance: These findings will facilitate further exploration of the pathophysiology of this condition and provide a robust means for assessing treatment effects in research settings.

Keywords:

Cirrhosis

EEG

Functional cortical networks

Hepatic encephalopathy

Pathophysiology

Psychometry

1. Introduction

The term 'hepatic encephalopathy' is used to describe the spectrum of neuropsychiatric change which commonly complicates the course of cirrhosis (Vilstrup et al., 2014). People with cirrhosis with clinically apparent deficits in mental and motor performance, for which there is no other discernible cause, are classified as having *overt* hepatic encephalopathy. Those with no apparent clinical neuropsychiatric deficits who nevertheless show neuropsychometric or neurophysiological abnormalities are classified as having *minimal* hepatic encephalopathy (Vilstrup et al., 2014). This condition, whether minimal or overt, significantly compromises patients' activities of daily living, their quality of life, their safety and their survival (Bustamante et al., 1999; Groeneweg et al., 1998; Roman et al., 2011; Schomerus et al., 1981). It also poses a substantial burden for carers (Bajaj et al., 2011) and a significant financial burden on healthcare systems (Stepanova et al., 2012).

The pathogenesis of hepatic encephalopathy is unknown although several important components of the complex underlying process have been identified (Häussinger and Sies, 2013). Ammonia plays a key role *via* induction of astrocyte swelling and the development of low-grade cerebral oedema. Astrocyte dysfunction results in alterations in gene expression, modification of proteins and RNA, and disturbances in intracellular signal transduction and neurotransmission. As a result glial-neuronal communication is disrupted and this is likely to impact on synaptic plasticity, oscillatory electric networks and, most likely brain connectivity (Häussinger and Sies, 2013).

Recently, resting-state functional magnetic resonance imaging (fMRI) has been used to study functional connectivity or the intrinsic coherence of neural activity, between distinct brain regions in patients with cirrhosis (Chen et al., 2013, 2015, 2016; Hsu et al., 2012; Jao et al., 2015; Qi et al., 2012). Overall, these studies have identified a 'stepwise' deterioration in functional brain networks with increasing degrees of psychometric impairment. However, the number of patients with hepatic encephalopathy included in these studies is limited. The exact numbers are unclear because there is acknowledged overlap of patients between studies but overall the number of fMRI studies undertaken in patients with cirrhosis, to date, is around 200; of these 104 (54%) were undertaken in patients with

minimal hepatic encephalopathy but only 16 (8%) in patients with overt hepatic encephalopathy (Hsu et al., 2012; Jao et al., 2015). Technical difficulties and costs are likely to be the main limiting factors. fMRI does not provide a direct measure of neuronal oscillations but rather an indirect measure based on changes in blood oxygenation, which may limit, to some extent, the interpretation of the findings.

Electroencephalography (EEG), in contrast to fMRI, provides direct information on cortical neuronal activity; it has been used to detect, assess and monitor hepatic encephalopathy since the 1950s (Foley et al., 1950; Marchetti et al., 2011; Montagnese et al., 2004; Parsons-Smith et al., 1957). The main electrophysiological characteristic of hepatic encephalopathy is slowing of the mean frequency from the alpha range (8.5-13 Hz) towards the theta (4-8 Hz) and delta (1-3.5 Hz) ranges (Jackson et al., 2016).

Recent developments in the field of EEG analysis and computational science have allowed investigation of neuronal networks *via* the functional connectivity between brain areas. This assessment is based on the phase-relationships between the EEG signals from spatially dispersed scalp electrodes representing cortical networks of synchronised neurons. Several methods exist to estimate functional connectivity based on the premise that electrodes with similar phase will exchange information (Fig. 1). The phase-lag index (PLI) is a relatively new method for assessing functional connectivity. In contrast to other methods, it obviates the 'common source problem' which may give rise to spurious correlations between time series (Nolte et al., 2008; Stam et al., 2007). Use of PLI may provide new insights into the cortical signature of hepatic encephalopathy and hence the pathogenesis of this syndrome.

The aims of this study were to investigate: i) EEG functional connectivity, and its cortical topography, in a cohort of patients with cirrhosis with or without neuropsychiatric impairment and to validate the findings in a second independent cohort; and, ii) the associations between EEG functional connectivity, psychometric performance, and the aetiology and severity of the underlying liver disease

2. Methods

2.1. The discovery population

The discovery population comprised of 264 patients with biopsy-proven cirrhosis (172 men: 92 women: mean [range] age 55.7 [24-80] years), recruited at the Royal Free Hospital, London between 2008 and 2016. The aetiology of the liver injury was determined using clinical, laboratory, radiological and histological variables; its severity was assessed using the model for end-stage liver disease (MELD) score and Pugh's modification of the Child's grading system (Pugh et al., 1973). Patients were excluded if they were <20 or >80 years of age; if they had hyponatraemia or renal failure; significant cardiac or respiratory failure; Type I diabetes mellitus or Type II diabetes with poor glycaemic control; cerebrovascular disease; epilepsy; a history of significant head injury or other conditions likely to affect cerebral function. Patients were also excluded if they had a surgically created or transjugular intrahepatic portal systemic shunt, had misused alcohol or drugs in the previous three months or were taking psychoactive medication. All patients were clinically stable at the time of the study.

2.2. The validation population

The validation population comprised of 106 patients with cirrhosis (72 men: 34 women; mean age 49.2 [19-69] years) recruited from the Department of Medicine in Hannover, Germany between 2008 and 2011. The exclusion criteria were those utilized for selection for liver transplantation which are broadly similar to those applied in UK patient cohort.

2.3. The healthy reference population

The healthy reference population comprised of 137 healthy individuals (73 men: 94 women; mean age 39.2 [17-75] years) recruited from family, friends and staff working at the Royal Free Hospital, London or else individuals who had experienced an isolated episode of fainting/dizziness but in whom clinical examination, the EEG, and cerebral imaging were normal at baseline and during prolonged follow-up. None had a history of liver disease, drank alcohol in excess of 20 g daily, or took prescription or over-the-counter medications.

2.4. Study procedures

Each UK participants was assessed in a single session lasting approximately two hours. Psychometric testing was undertaken in a quiet well-lit room; tests were presented in the same order, using a standard set of instructions from scripted texts. The EEGs were performed in a dedicated recording room by a trained neurophysiologist. The German participants were assessed using similar procedures and test conditions to those used in the UK.

2.4.1. Psychometric assessment:

Psychometric performance was assessed using the Psychometric Hepatic Encephalopathy Score (PHES) battery (Weissenborn et al., 2001), which comprises of five paper and pencil tests *viz*: number connection A and B; digit symbol; serial dotting; and line tracing.

The raw PHES data collected in the UK were adjusted and scored using UK normative values; composite scores of less than two standard deviations below mean reference values were considered abnormal (Marks et al., 2008). For purposes of this study patients were ranked by their composite final score as follows: PHES >0; PHES 0 to -2; PHES -2 to -4; and PHES <-4 (Marks et al., 2008).

The raw PHES data collected in Germany were adjusted and scored using German normative reference data (Weissenborn et al., 2001); the final PHES score comprised the sum of Z-scores of the individual tests and was considered abnormal if less than -4. For purposes of this study patients were ranked by their composite final score as follows: PHES >0; PHES 0 to -4; PHES -4 to -8; and PHES <-8.

2.4.2. EEG recording:

EEGs were recorded, in the UK, using one of two digital EEG systems *viz.* Walter-Graphtek PL-Winsor (Walter-Graphtek GmbH, Emmendingen, Germany) or MicroMed SystemPlus EVOLUTION (Micromed Sp.A., Mogliano, Veneto, Italy). Recordings were undertaken for 6-10 min, in a state of eyes-closed, relaxed, wakefulness, using 23 silver-silver chloride electrodes placed according to the International 10-20 System. The sampling rate was 256 Hz/s with the online band filter set at 0.05 to 70.0 Hz. The impedance of the electrodes was kept below 5 KΩ.

EEGs were recorded in Germany using a Walter-Graphtec digital EEG recording system (Walter-Graphtek GmbH, Emmendingen, Germany), under similar conditions to those used in the UK. The data were exported and dispatched to the Department of Clinical Neurophysiology, at the Royal Free London NHS Foundation Trust, for further computational processing.

2.4.3. EEG analysis:

2.4.3.1. *EEG pre-processing*: A consecutive, 60–100s of eyes-closed, artefact-free recording was selected from the P3-P4 derivation. If the length of artefact-free recording available for analysis was insufficient, the selection criteria were relaxed to allow use of a combination of artefact-free sections from the same derivation.

2.4.3.2. Spectral EEG analysis: The preselected 60–100s of recording was divided into two second epochs and multiplied with a cosine window with a one second overlap. Fast Fourier transform was applied to obtain the spectral power in each 0.5 Hz bin; this was averaged across all epochs to give an average power spectrum for each EEG. Spectral estimates were obtained for the mean dominant frequency (MDF), which is the weighted mean of frequencies in the 1 to 26.5 Hz range, and the relative power percentages in the standardized frequency bands *viz*: delta (1–3.5 Hz), theta (4–8 Hz), alpha (8.5–13 Hz) and beta (13.5–26.5 Hz) (Montagnese et al., 2007).

2.4.3.3. Functional connectivity analysis: The PLI was calculated using the Neurophysiological Biomarker Toolbox (NBT) (http://www.nbtwiki.net/) on a 19 channels average reference montage based of the 10-20 System omitting A1 and A2 mastoid electrodes. The EEG signal was band passed into four frequency bands *viz*: delta (1–3.5 Hz), theta (4–8 Hz), alpha (8.5–13 Hz) and beta (13.5–32.0 Hz): using first-order Butterworth filters (Lehembre et al., 2012) and was then divided into two second epochs. The PLI was calculated for each epoch as an asymmetry index of the phase difference distribution obtained from the EEG signal (Stam et al, 2007); individual epoch values were then averaged to provide a measure of functional connectivity for the full EEG recording. PLI values range between 0 and 1. A PLI of 0 indicates

either no coupling or coupling with a phase difference centered on 0. A PLI of 1 indicates perfect phase locking; the stronger the non-zero phase locking the larger the PLI.

2.5. Data processing: creation of the discovery and validation cohorts

Healthy reference data were not collected in Germany. Thus, the 137 EEGs collected from healthy individuals in the UK were divided to provide independent control data sets for both the UK and German patient populations. The 137 EEGs were first ordered by sex and EEG MDF and then individually assigned to either the discovery or the validation cohorts, maintaining the same proportionate distribution as in the patients. Thus, the discovery cohort comprised of 264 patients and 98 healthy controls while the validation cohort comprised of 106 patients and 39 healthy controls.

2.6. Statistical analysis

The distribution of variables was assessed by visual inspection of QQ-plots; the assumption of variance homogeneity was checked using Bartlett's test. The potential confounding effects of age and sex on functional connectivity variables were assessed in the reference population using linear regression and the necessary adjustments applied to the patients' connectivity variables prior to statistical analysis. Differences between normally distributed spectral EEG variables, clinical and demographic characteristics as well as psychometric tests were examined by one-way ANOVA and Chi-squared tests, as appropriate. Differences between non-normally distributed variables were examined using the Kruskal-Wallis test or Dunn's pair wise comparison test.

To aid interpretation of the topographical information contained in the multichannel EEG, average functional connectivity was calculated for five distinct cortical regions *viz*. frontal, temporal, central, parietal and occipital, by averaging the connectivity estimates from the corresponding EEG electrodes. The association between functional connectivity and psychometric test performance based on ranking of the final composite PHES score was assessed using a linear mixed effects models fitted by maximum likelihood estimation, topographic information was included in the models as a random effect. The association between functional connectivity, psychometric performance, assessed by the raw PHES score, and the severity and aetiology of the underlying liver disease was assessed using linear mixed

effect models; topographical information was again included as a random effect in the models.

Pearson's coefficient was used for correlation analysis of functional connectivity and spectral EEG estimates. Reported P-values were two-tailed; values ≤0.05 were considered statistically significant; the Bonferroni adjustment was applied for the topographical analysis to account for multiple comparisons. The software packages STATA version 14.1 (StataCorp LP, College Station, Texas) and R version 3.0.2 (CRAN.R-project.org/doc/FAQ/R-FAQ.html) were utilized.

2.7. Ethical considerations

The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines). The protocol was approved by the Royal Free Hampstead NHS Trust Ethics Committee, London UK and the Ethics Committee of Hannover Medical School, Hannover, Germany. All participating subjects, or their appropriately appointed guardian, provided written, informed consent.

3. Results

3.1. The discovery cohort

The aetiology of the cirrhosis was alcohol in 177 (67.1%) of the 264 patients, alcohol and hepatitis C virus (HCV) infection in 21 (8.0%), cryptogenic in 14 (5.3%), fatty liver disease in 16 (6.0%), HCV/HBV infection in ten (3.8%), primary biliary cholangitis in six (2.3%), autoimmune hepatitis in eight (3.0%), haemochromatosis in four (1.5%) and 'other aetiologies' in eight (3.0%) (Table 1). On the day of study 42 (15.9%) patients had a PHES score >0; 132 (50.0%) a score of 0 to -2; 75 (28.4%) a score of -2 to -4 and 15 (5.7%) a score of <-4 (Table 1). The degree of hepatic decompensation, evidenced by the MELD scores, increased with the degree of psychometric impairment (Table 1).

3.2. EEG analysis

A total of 264 EEGs were recorded in the patient discovery population and 98 in the reference population. None of the EEGs was discarded because of abnormal focal activity; analysable data were available from all recorded EEGs.

3.2.1. Spectral EEG estimates

Progressive abnormalities of the EEG were observed in the patients with cirrhosis with increasing psychometric impairment (Supplementary Table 1); this was characterized by overall slowing as a result of increased low frequency (delta and theta) activity and decreased high frequency (alpha and beta) activity. The most significant changes were observed in the patient with a PHES score of <-2.

3.2.2. Functional connectivity

Mean connectivity in the theta band was significantly higher in the patients with cirrhosis than the reference population, whilst mean connectivity in the delta, alpha and beta band was significantly decreased (Table 2). Significant correlations were observed between spectral EEG and functional connectivity parameters in all but the beta band (Pearson`s correlation coefficients 0.35-0.70; $p<0.001$); the strongest relationships were observed in the theta and alpha bands (Supplementary Table 2).

3.3. Functional connectivity, by psychometric performance

Widespread differences were observed in functional connectivity in the patients with cirrhosis, across frequency bands, in relation to psychometric performance (Fig. 2; Table 2). No significant changes were observed in the subgroup of patients with a PHES score >0 but significant changes were apparent in the subgroup with PHES scores between 0 and -2. More pronounced changes, characterized by increased theta connectivity and decreased connectivity in the remaining frequency bands were observed with increasing psychometric impairment; the exception being a ´secondary increase´ of delta connectivity in patients with a PHES score of <-4 (Fig. 2; Table 2).

3.4. Functional connectivity by psychometric performance and EEG topography

There were significant topographical differences between functional connectivity parameters in the patients with cirrhosis in relation to their psychometric performance (Supplementary Table 3). The most prominent feature was the gradual replacement of frontal connectivity in the alpha band with increasing frontal connectivity in the theta band as the degree of psychometric impairment increased (Fig. 3).

3.5. Univariate and multivariate analyses for associated variables

On univariate analysis, functional connectivity was progressively reduced in the alpha and beta bands with increasing psychometric impairment, while an inverse relation was seen with the theta band (Table 3). The MELD score was significantly associated with functional connectivity in the theta and alpha bands, but there were no associations between functional connectivity and the aetiology of the liver disease in any of the frequency bands (Table 3).

Multivariate analysis confirmed the independence and significance of these associations *viz.* PHES score and functional connectivity in the theta band (coefficient = -0.003; p <0.001), alpha band (coefficient = 0.007; p<0.001) and beta band (coefficient = 0.001; p<0.001); MELD score and functional connectivity in the alpha band (coefficient -0.001; p<0.001) (Table 3).

3.6. The validation cohort

The aetiology of the liver disease in the 106 German patients was predominantly non-alcohol-related *viz* HCV infection in 38 (35.8%); primary sclerosing cholangitis in 23 (21.7%); cryptogenic in 11 (10.4%); chronic HBV infection in six (5.7%); polycystic liver disease in five (4.7%); chronic active hepatitis in four (3.8%); alcohol in two (1.9%) and 'other aetiologies' in 17 (16.0%). The mean MELD score was 12.3 (5.6-23.6). On the day of study 30 (28.3%) patients had a PHES score >0; 47 (44.3%) a score of 0 to -4; 16 (15.1%) a score -4 to -8 and 13 (12.3%) a score of <-8.

A total of 106 EEGs were recorded in the patient validation population and 39 in the allocated reference population. None of the EEGs was discarded because of abnormal focal activity; analysable data were available from all the recorded EEGs.

The widespread changes in functional connectivity observed in the discovery population, in relation to psychometric performance, were replicated in the validation population (Fig. 4; Supplementary Table 4). In particular: (i) changes in functional connectivity within a subgroup of patients in whom psychometric performance would ordinarily be considered unimpaired *viz* PHES score 0 to -4; and, (ii) the progressive increase in theta connectivity and further decreases in connectivity in the remaining frequency bands with increasing psychometric impairment, except, in this instance, within the delta band (Fig. 4; Supplementary Table 4).

4. Discussion

In the present study, brain networks were investigated, using multichannel EEG and the surrogate variable functional connectivity, in a large well-characterized population of patients with cirrhosis with varying degrees of neuropsychometric impairment. Widespread disruption was observed even in patients in whom psychometric performance was unimpaired; further disruption was observed with increasing deterioration in psychometric performance. The disruption in functional connectivity was positively associated with the severity of the underlying liver disease but not its aetiology. The main findings were validated in a second independent cohort of patients; in particular the presence of disrupted functional connectivity in the absence of psychometric impairment was again observed, as was the progression of the disruption with increasing psychometric impairment. Moderate to strong correlations were observed between spectral EEG and functional connectivity variables, particularly in the theta and alpha bands, but these were not absolute. Thus, the assessment of connectivity provides complementary information not captured by conventional EEG examination based on spectral analysis.

4.1. Functional connectivity and psychometric performance

A clear and progressive disruption in functional connectivity was observed, in the patients with cirrhosis, with increasing psychometric impairment. There are no comparable EEG studies with which to compare these findings. Marchetti *et al*. (2011) examined EEG coherence, as a measure of functional interaction, in a large group of patients with cirrhosis and found an association between increasing cognitive impairment and higher frontal-to-parietal and posterior inter-hemispheric coherence within the theta band. However, coherence is sensitive to volume conduction through the cortical membranes, scalp and skin, which may bias the assessment (Marchetti et al., 2011; Stam et al., 2007)

Functional cortical connectivity has been studied in patients with cirrhosis and varying degrees of psychometric impairment using resting state fMRI (Chen et al., 2013, 2015, 2016; Hsu et al., 2012; Jao et al., 2015; Qi et al., 2012). The results of these studies, which were undertaken in China and Taiwan,

have been published in serial papers with acknowledged overlap of patients between studies. The majority of included patients had hepatitis B-related cirrhosis and their neuropsychiatric status was assessed simply by clinical examination and use of a small number of psychometric tests.

The Chinese group confined their studies to patients with cirrhosis who were psychometrically unimpaired or who had minimal hepatic encephalopathy (Chen et al., 2013, 2015, 2016; Qi et al., 2012). Neurocognitive networks were found to be atypical (Chen et al., 2016) with a progressive trend for increased disruption with worsening psychometric performance (Chen et al, 2013, 2015, 2016; Qi et al., 2012). The effects showed regional specificity (Qi et al., 2012) and regional heterogeneity in relation to their correlation with psychometric performance (Chen et al., 2013).

The Taiwanese group also found disrupted brain network organization in patients with cirrhosis and an increase in the network disruption with greater neuropsychometric impairment (Hsu et al., 2012; Jao et al., 2015). Similar functional network randomization has also been observed in conditions such as Alzheimer's disease (Stam et al., 2009), epilepsy (van Dellen et al., 2009), and traumatic brain injury (Nakamura et al., 2009), suggesting that it might represent a final common pathway for several brain pathologies. Thus, this feature would appear to be a sensitive marker of neuropsychiatric disease, but it lacks specificity. The Taiwanese group also reported that patients with cirrhosis show a reduction in both global and nodal connectivity strength; this is a feature of brain disintegration and has been observed in both fMRI and EEG studies in propofol-induced anaesthesia (Schröter et al.,2012), analgesia with sedation (Khodayari-Rostamabad et al., 2015), sleep (Massimini et al., 2005), and coma (Crone et al., 2018).

Thus overall the results of the fMRI studies confirm the findings in the present study that cortical networks are disrupted in patients with cirrhosis and that the degree of disruption correlated with the degree of psychometric impairment.

4.2. Functional connectivity and the severity and aetiology of the liver disease

The degree of disruption of functional connectivity observed in the present study increased with

deteriorating liver function. In the multivariate analysis, the MELD score was independently associated with the functional EEG connectivity measures in the alpha band. This is to be expected as hepatic encephalopathy is associated with, and is a feature of, a reduction in hepatic functional reserve. Marchetti et al., (2011), also found a significant relationship between EEG coherence and the degree of liver injury.

No association was observed between the aetiology of liver disease and functional EEG connectivity parameters. This contrast with previous EEG studies which showed an increase in beta activity in alcohol-related cirrhosis, possibly reflecting changes in gamma aminobutyric acid (GABA) neurotransmission (Olesen et al., 2016). Variations in the GABA alpha-2 gene are strongly associated with the beta frequency of the human EEG and genetic studies have provided evidence of involvement of the GABA system in the susceptibility to develop alcohol dependence through an effect on neural excitability (Lydall et al., 2011; Saletu-Zyhlarz et al., 2004). However, alcohol *per se* modulates GABAergic neurotransmission resulting in an increase in the beta activity in the EEG in people actively consuming alcohol (Anstee et al., 2013). Thus, spectral EEG estimates must be evaluated carefully in the context of alcohol-related cirrhosis; the increased activity in the high frequency range observed in this patient group may compromise the reliability and validity of spectral thresholds (Olesen et al., 2016). The functional EEG measures presented in the present study are likely to provide a more robust and reliable tool for assessment of the neurophysiological abnormalities associated with hepatic encephalopathy in the context of alcohol-related cirrhosis. This was further illustrated by the lack of association between spectral and functional connectivity parameters in the beta band.

4.3. Study strengths and limitations

This study has a number of strengths: (i) the discovery population was large and well-characterized and comprised of patients with varying degrees of neuropsychometric impairment. This allowed for detailed stratification and analysis of subgroups with documentation of subtle changes in brain function that would not have been detected using psychometric testing and conventional normative thresholds. The large sample sizes, in addition, allowed for a detailed analysis of possible confounding

factors including age, sex and the severity and aetiology of the liver Injury; (ii) an independent validation population of equally well-characterized patients was included allowing the main study findings to be ratified; and, (iii) the technique used for assessing functional connectivity is not sensitive to volume conduction which is a problem with other assessment techniques, such as, coherence.

The study is also limited primarily because: (i) its cross-sectional design precludes causal inference about the relationship between psychometric performance and the functional EEG measures; (ii) it was not designed to determine when the changes in functional connectivity translate into clinically meaningful deficits in neuropsychiatric performance.

In conclusion: cortical networks are disturbed in patients with cirrhosis even in the absence of psychometric impairment; these findings could provide further insights into the pathophysiology of the hepatic encephalopathy and a robust and objective mean for assessing treatment effects in a research setting.

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Legends to Figures:

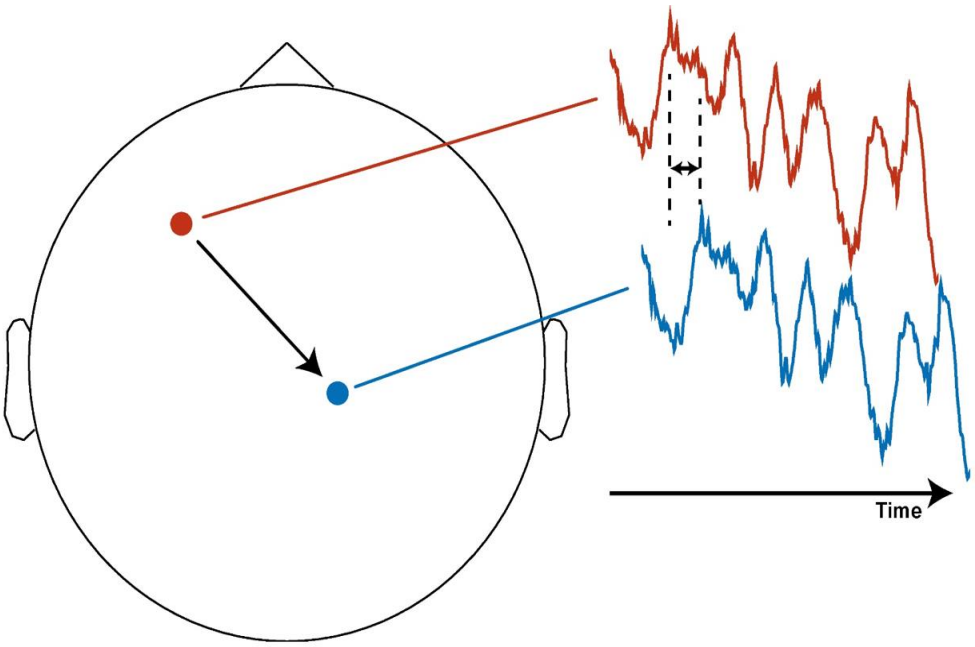

Figure 1: The principle behind measurement of functional connectivity using the phase-lag index

The signal from the receiving electrode (blue) is phase-shifted compared to the signal from the driving electrode (red). This indicates a strong connection between the two electrodes and hence networks of synchronized neurons.

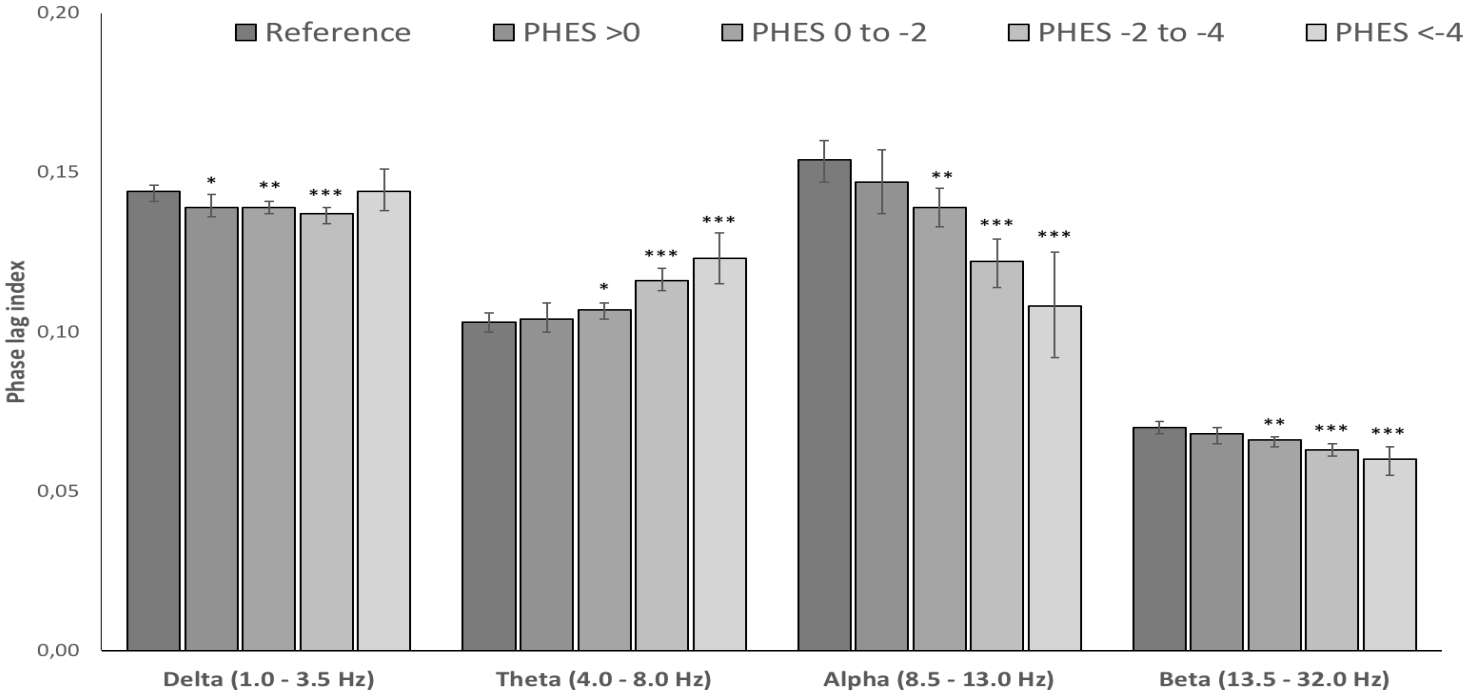

Figure 2: Electroencephalogram functional connectivity in the discovery cohort, by neuro-psychometric performance, stratified by the PHES score

Data are expressed as estimates from linear mixed models ± SEM

PHES Psychometric Hepatic Encephalopathy Score

Significance of the difference between the reference population and the various patient subgroups;

* p<0.05; ** p<0.01; *** p<0.001

The significances of the differences between the subgroups of patients with by PHES score are not indicated (see Table 2 for details)

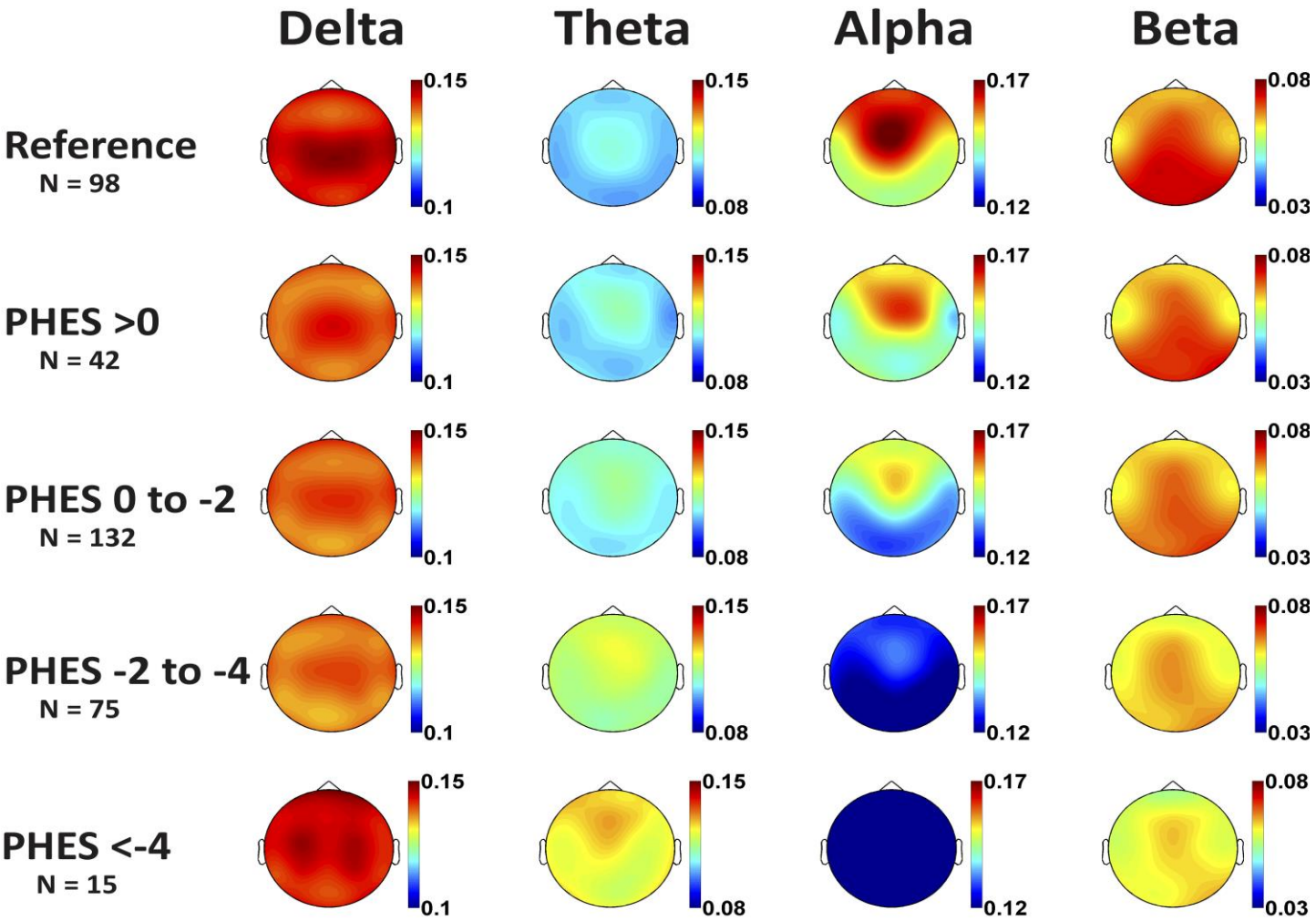

Figure 3: **Average head maps showing the spatial distribution of functional connectivity estimates in the discovery cohort, by neuropsychometric performance, stratified by the PHES score**

The raw PHES data were adjusted and scored using UK normative values; a PHES score <-2 was considered abnormal using conventional thresholds.

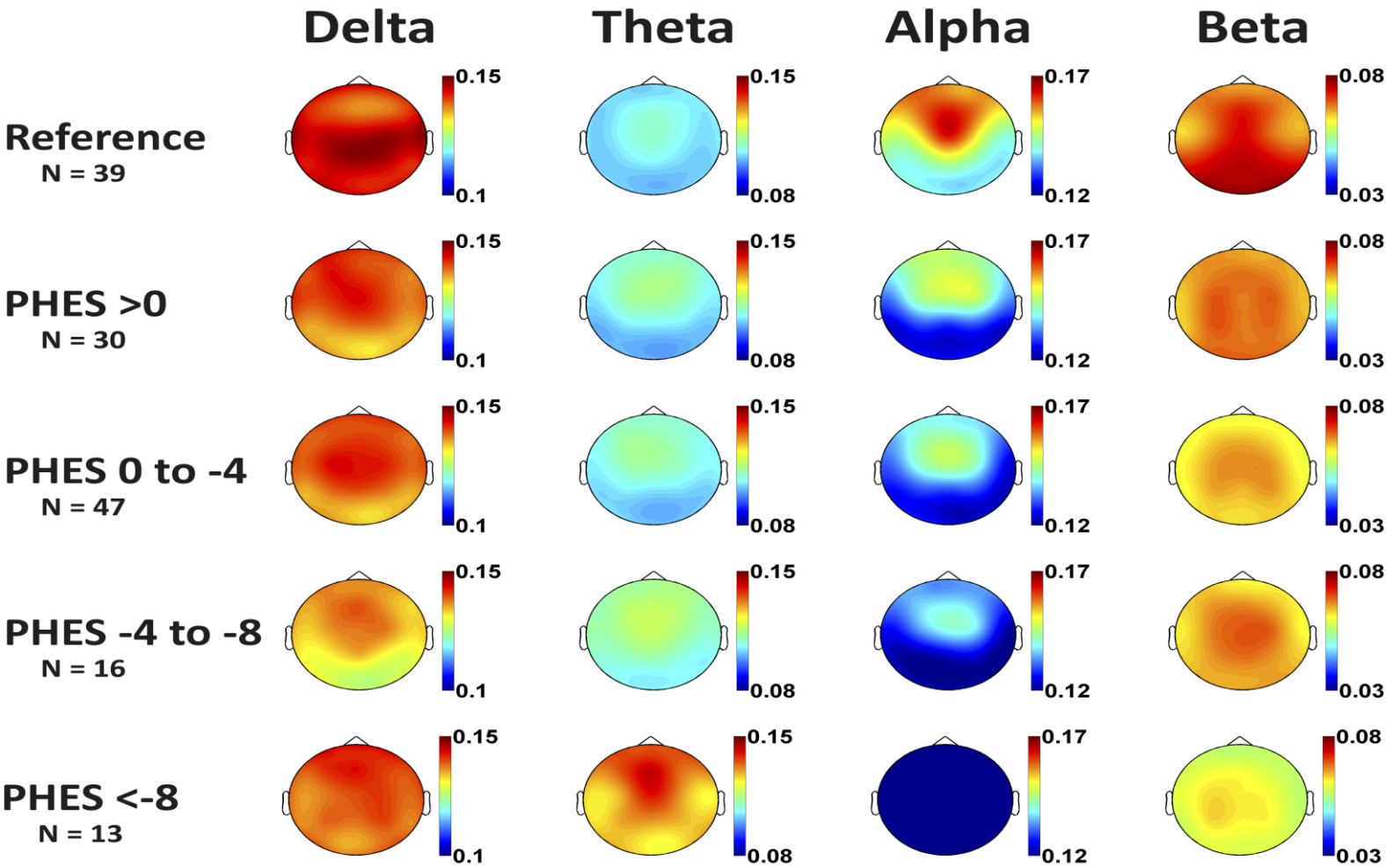

Figure 4: Average head maps showing the spatial distribution of functional connectivity estimates in the validation cohort, by neuropsychometric performance, stratified by the PHES score

The raw PHES data were adjusted and scored using German normative values; a PHES score <-4 was considered abnormal using conventional thresholds.

Table 1: Demographic and assessment variables in the patient discovery population, by performance on the PHES battery and its components

Study population (n)	Age (yr)	Male n (%)	MELD score	NCT A (s)	NTC B (s)	DS (n correct)	SD (s)	LTT (s)	LTE (n errors)	PHES score[€]
Reference (98)	39.6±15.5 (17-75)	52 (53.1)								
Cirrhosis: All (264)	55.7±10.1$^{\wedge\wedge\wedge}$ (24-81)	172$^{\wedge}$ (65.2)	13.1±5.7 (6-36)	54.8±46.7 (16-480)	149.2±103.7 (22-480)	34.8±13.7 (0-80)	68.5±36.6 (29-333)	104.5±56.2 (25-431)	60.5±59.1 (0-389)	-1.5±1.5 (-7 to 2)
Cirrhosis: PHES >0 (42)	54.4±8.9$^{\wedge\wedge}$ (39-77)	27 (64.3)	10.1±3.1 (6-19)	27.8±8.9 (16-68)	61.8±24.4 (22-148)	51.6±8.7 (30-80)	41.6±7.6 (29-65)	71.7±26.6 (25-145)	39.6±35.3 (3-138)	0.6±0.5 (0 to 2)
Cirrhosis: PHES 0 to -2 (132)	55.1±10.2$^{\wedge\wedge}$ (24-81)	90 (68.2)	12.7±5.9** (6-32)	42.4±16.7** (18-152)	113.0±57.7** (42-400)	38.0±9.1** (15-63)	57.2±17.1** (31-111)	90.6±35.9* (25-213)	47.4±38.1 (2-197)	-1.0±0.5** (-2 to 0)
Cirrhosis: PHES -2 to -4 (75)	57.5±11.3$^{\wedge\wedge}$ (26-81)	46 (61.3)	14.4±5.8$^{**\#}$ (6-36)	69.1±32.4$^{**\#\#}$ (30-233)	223.4±104.7$^{**\#\#}$ (73-480)	24.4±8.1$^{**\#\#}$ (0-42)	84.7±28.4$^{**\#\#}$ (48-202)	125.2±55.6$^{**\#\#}$ (41-360)	71.9±52.8$^{**\#}$ (0-211)	-2.8±0.5$^{**\#\#}$ (-4 to-2)
Cirrhosis: PHES <-4 (15)	55.3±6.3$^{\wedge\wedge}$ (46-69)	9 (60.0)	18.4±4.4$^{**\#\#\&}$ (13-26)	167.5±119.5$^{**\#\#\&}$ (64-480)	342.3±79.4$^{**\#\#}$ (220-480)	11.7±7.4$^{**\#\#\&\&}$ (3-29)	162.6±60.8$^{**\#\#\&}$ (83-333)	214.4±92.7$^{**\#\#\&}$ (103-431)	177.8±123.5$^{**\#\#\&}$ (35-389)	-4.9±0.8$^{**\#\#\&\&}$ (-7 to -4)

Data are expressed as mean ± 1SD (range) or absolute number (%)

HE: hepatic encephalopathy; MELD: model for end-stage liver disease; NCT-A/B, Number Connection Tests A/B; DS: Digit Symbol Test; LT-T/E: Line Tracing Test-Time/Errors; SD: Serial Dotting Test; PHES Psychometric Hepatic Encephalopathy Score

[€]PHES score range for UK identification cohort is standardized so the original normative dataset had a mean of zero and a SD of one

Significance of the difference between the reference population and the various patient subgroups; $^{\wedge}$ p<0.05; $^{\wedge\wedge}$ p<0.01; $^{\wedge\wedge\wedge}$ p<0.001
Significance of the difference between patients with a PHES score >0 and those with a PHES score 0 to -2; * p<0.05; ** p<0.001
Significance of the difference between patients with a PHES score 0 to -2 and those with a PHES score -2 to -4; $^{\#}$ p<0.05; $^{\#\#}$ p<0.001
Significance of the difference between patients with a PHES score -2 to -4 and those with a PHES score <-4; $^{\&}$ p<0.05; $^{\&\&}$ p<0.001

Table 2: Electroencephalogram functional connectivity in the patient discovery population, by PHES performance

Study population (n)	**Delta (1-3.5 Hz)**	**Theta (4-8 Hz)**	**Alpha (8.5-13 Hz)**	**Beta (13.5-32.0 Hz)**
Reference population (98)	0.144±0.001 (0.142-0.147)	0.103±0.002 (0.100-0.106)	0.154±0.003 (0.148-0.161)	0.070±0.001 (0.068-0.072)
Cirrhosis: all (264)	0.139±0.001$^{\wedge\wedge\wedge}$ (0.137-0.140)	0.110±0.001$^{\wedge\wedge\wedge}$ (0.108-0.112)	0.134±0.002$^{\wedge\wedge\wedge}$ (0.130-0.138)	0.065±0.001$^{\wedge\wedge\wedge}$ (0.064-0.066)
Cirrhosis: PHES score >0 (42)	0.139±0.002$^{\wedge}$ (0.136-0.143)	0.104±0.002 (0.100-0.109)	0.147±0.005 (0.137-0.157)	0.068±0.001 (0.065-0.070)
Cirrhosis: PHES score 0 to -2 (132)	0.139±0.001$^{\wedge\wedge}$ (0.137-0.141)	0.107±0.001$^{\wedge}$ (0.105-0.110)	0.139±0.003$^{\wedge\wedge}$ (0.133-0.145)	0.066±0.001$^{\wedge\wedge}$ (0.065-0.068)
Cirrhosis; PHES score -2 to -4 (75)	0.137±0.001$^{\wedge\wedge\wedge}$ (0.135-0.140)	0.116±0.002$^{\wedge\wedge\wedge **\#\#\#}$ (0.112-0.119)	0.122±0.004$^{\wedge\wedge\wedge **\#\#\#}$ (0.115-0.130)	0.063±0.001$^{\wedge\wedge\wedge *\#\#}$ (0.061-0.065)
Cirrhosis: PHES score <-4 (15)	0.144±0.003 (0.137-0.150)	0.123±0.004$^{\wedge\wedge\wedge **\#\#\#}$ (0.115-0.131)	0.108±0.008$^{\wedge\wedge\wedge **\#\#\#}$ (0.091-0.124)	0.060±0.002$^{\wedge\wedge\wedge *\#}$ (0.056-0.065)

Data are expressed as estimates from linear mixed models ± SEM (95% CI)

PHES Psychometric Hepatic Encephalopathy Score

Significance of the difference between the reference population and the various patient subgroups; ^ $p<0.05$; ^^ $p<0.01$; ^^^ $p<0.001$
Significance of the difference between patients with PHES score (>0) and the other patient subgroups; * $p<0.01$ ** $p<0.001$
Significance of the difference between patients with PHES score (0 to -2) and the other patient subgroups; $^{\#}$ $p<0.05$; $^{\#\#}$ $p<0.01$; $^{\#\#\#}$ $p<0.001$

Table 3: Univariate and multivariate linear mixed effect models of functional cortical connectivity against psychometric performance and liver Disease aetiology and severity, by frequency bands

Frequency band	Assessment variable	Univariate		Multivariate	
		Coefficient	P-value	Coefficient	P-value
Delta (1-3.5 Hz)	**Psychometric performance (PHES)**	-0.0007±0.0005	0.16	-0.0010±0.0005	0.048
	Liver disease severity (MELD)	-0.0001±0.0001	0.34	-0.0003±0.0001	0.011
	Liver disease aetiology (alcohol/non-alcohol)	0.0009±0.0017	0.59	0.0005±0.0016	0.74
Theta (4-8Hz)	**Psychometric performance (PHES)**	-0.0039±0.0007	<0.001	-0.0032±0.0008	<0.001
	Liver disease severity (MELD)	0.0008±0.0002	<0.001	0.0004±0.0002	0.058
	Liver disease aetiology (alcohol/non-alcohol)	-0.0002±0.0027	0.94	-0.0010±0.0025	0.69
Alpha (8.5-13 Hz)	**Psychometric performance (PHES)**	0.0084±0.0013	<0.001	0.0066±0.0013	<0.001
	Liver disease severity (MELD)	-0.0020±0.0003	<0.001	-0.0013±0.003	<0.001
	Liver disease aetiology (alcohol/non-alcohol)	0.0058±0.0047	0.22	0.0053±0.0041	0.20
Beta (13.5-32 Hz)	**Psychometric performance (PHES)**	0.0011±0.0003	<0.001	0.0011±0.0003	<0.001
	Liver disease severity (MELD)	-0.0001±0.001	0.066	-0.0001±0.0001	0.96
	Liver disease aetiology (alcohol/non-alcohol)	0.0012±0.0001	0.22	0.0006±0.0009	0.50

Data are expressed as estimates from linear mixed effect models ± SEM

Supplementary Table 1: Electroencephalogram spectral estimates in the patient discovery population, by PHES performance

Study population (n)	MDF	Delta (1-3.5 Hz)	Theta (4-8 Hz)	Alpha (8.5-13 Hz)	Beta (13.5-26.5 Hz)
Reference population (98)	9.8 ± 1.4	17.6 ± 9.2	16.0 ± 6.6	45.8 ± 16.8	20.6 ± 11.4
Cirrhosis: all (264)	9.5 ± 2.0	16.9 ± 12.2	25.7 ± 15.6	36.6 ± 17.5	20.8 ± 12.5
Cirrhosis: PHES >0 (42)	10.3±1.4	14.1±6.2	18.8±11.9	42.9±13.4	24.2±10.4
Cirrhosis: PHES 0 to -2 (132)	10.0±1.7	14.6±9.3$^{\wedge}$	21.7±12.9$^{\wedge}$	40.6±17.0	23.1±12.4
Cirrhosis: PHES -2 to -4 (75)	8.7±2.0$^{\wedge\wedge***\#\#}$	18.3±12.5	34.5±17.6$^{\wedge\wedge***\#\#}$	30.2±16.6$^{\wedge\wedge***\#\#}$	17.0±12.5$^{*\#}$
Cirrhosis: PHES< -4 (15)	6.4±2.5$^{\wedge\wedge***\#\#}$	38.9±21.6$^{\wedge\wedge\#\#***€€}$	35.4±13.0$^{\wedge\wedge***\#}$	14.8±10.0$^{\wedge\wedge***\#\#€}$	10.9±10.1$^{\wedge**\#}$

Data are expressed as means ± 1 SD

PHES Psychometric Hepatic Encephalopathy Score

Significance of the difference between the reference population and the various patient subgroups; ^ p<0.05; ^^ p<0.001
Significance of the difference between patients with PHES score (0 to -2) and the other patient subgroups; * p<0.01; ** p<0.001
Significance of the difference between patients with PHES score (-2 to – 4) and the other patient subgroups; # p<0.01; ## p<0.001
Significance of the difference between patients with PHES score <-4 and the other patient subgroups; € p<0.05; €€ p<0.001

Supplementary Table 2: Correlation between the electroencephalogram spectral estimates (P3-P4 derivation) and functional connectivity, by EEG topography

Cortical region	Delta	Theta	Alpha	Beta
Grand mean	**0.42**	**0.68**	**0.65**	-0.001
Frontal	**0.46**	**0.70**	**0.64**	0.03
Temporal	**0.35**	**0.68**	**0.63**	-0.02
Central	**0.37**	**0.65**	**0.63**	0.04
Parietal	**0.39**	**0.57**	**0.63**	-0.02
Occipital	**0.39**	**0.60**	**0.63**	-0.07

Values in bold typeface are significant, $p<0.001$

Supplementary Table 3: Electroencephalogram functional connectivity in the patient discovery population, by psychometric performance and EEG topography

Data are

Region	Population group	Delta (1-3.5 Hz)	Theta (4-8 Hz)	Alpha (8.5-13 Hz)	Beta (13.5-32 Hz)
Frontal	**Reference population**	0.141±0.001	0.103±0.002	0.162±0.003	0.066±0.001
	Cirrhosis: PHES >0	0.138±0.002	0.105±0.002	0.152±0.005	0.064±0.001
	Cirrhosis: PHES 0 to -2	0.139±0.001	0.110±0.001^	0.148±0.003^	0.063±0.001
	Cirrhosis: PHES -2 to -4	0.137±0.001	0.119±0.002^^^***##	0.128±0.004^^^**###	0.061±0.001^^
	Cirrhosis: PHES< -4	0.145±0.003	0.126±0.004^^^***##	0.111±0.009^^^***##	0.059±0.002^
Temporal	**Reference population**	0.145±0.001	0.101±0.002	0.147±0.003	0.066±0.001
	Cirrhosis: PHES >0	0.138±0.002^	0.102±0.002	0.140±0.005	0.065±0.001
	Cirrhosis: PHES 0 to -2	0.139±0.001^^	0.105±0.001	0.135±0.003	0.064±0.001
	Cirrhosis: PHES -2 to -4	0.137±0.001^^^	0.114±0.002^^^***##	0.121±0.004^^^*#	0.061±0.001^^^
	Cirrhosis: PHES< -4	0.142±0.003	0.123±0.004^^^***###	0.108±0.009^^^*#	0.059±0.002^
Central	**Reference population**	0.146±0.001	0.106±0.002	0.164±0.003	0.070±0.001
	Cirrhosis: PHES >0	0.142±0.002	0.108±0.002	0.156±0.005	0.068±0.001
	Cirrhosis: PHES 0 to -2	0.141±0.001^	0.110±0.001	0.147±0.003^^	0.067±0.001
	Cirrhosis: PHES -2 to -4	0.140±0.001^^	0.119±0.002^^^**##	0.127±0.004^^^***##	0.064±0.001^^
	Cirrhosis: PHES< -4	0.146±0.003	0.124±0.004^^**#	0.110±0.009^^^***##	0.062±0.002^^
Parietal	**Reference population**	0.147±0.001	0.105±0.002	0.152±0.003	0.074±0.001
	Cirrhosis: PHES >0	0.141±0.002	0.104±0.003	0.144±0.005	0.071±0.002
	Cirrhosis: PHES 0 to -2	0.139±0.001^^	0.107±0.001	0.135±0.003^^	0.068±0.001^^
	Cirrhosis: PHES -2 to -4	0.138±0.002^^^	0.115±0.002^^^**#	0.119±0.004^^^**##	0.065±0.001^^^*
	Cirrhosis: PHES< -4	0.144±0.004	0.121±0.004^^*#	0.105±0.009^^^**#	0.062±0.003^^^*
Occipital	**Reference population**	0.142±0.002	0.099±0.002	0.145±0.003	0.074±0.001
	Cirrhosis: PHES >0	0.137±0.002	0.103±0.003	0.142±0.005	0.072±0.002
	Cirrhosis: PHES 0 to -2	0.136±0.001^	0.104±0.001	0.130±0.003^	0.069±0.001^^
	Cirrhosis: PHES -2 to -4	0.136±0.002	0.112±0.002^^^*##	0.115±0.004^^^**#	0.064±0.001^^^**#
	Cirrhosis: PHES< -4	0.141±0.004	0.120±0.004^^^**##	0.104±0.009^^^**	0.061±0.003^^^*

expressed as estimates from linear mixed models ± SEM; PHES Psychometric Hepatic Encephalopathy Score;
Bonferroni adjustments applied to account for multiple testing
Significance of the difference between the reference population and the various patient subgroups; ^ p<0.05; ^^ p<0.01; ^^^p<0.001
Significance of the difference between patients with PHES score (>0) and the other patient subgroups; * p<0.05; ** p<0.01 *** p<0.001
Significance of the difference between patients with PHES score (0 to – 2) and the other patient subgroups; # p<0.05; ## p<0.01; ### p<0.001

Supplementary Table 4: Electroencephalogram functional connectivity in the patient validation population, by psychometric performance

Study population (n)	**Delta (1-3.5 Hz)**	**Theta (4-8 Hz)**	**Alpha (8.5-13 Hz)**	**Beta (13.5-32.0 Hz)**
Reference population (39)	0.144±0.002 (0.140-0.148)	0.104±0.002 (0.100-0.108)	0.147±0.005 (0.138-0.157)	0.071±0.001 (0.069-0.074)
Cirrhosis: all (106)	0.138±0.001$^{\wedge\wedge}$ (0.135-0.140)	0.109±0.001 (0.107-0.112)	0.129±0.003$^{\wedge\wedge}$ (0.123-0.134)	0.064±0.001$^{\wedge\wedge\wedge}$ (0.063-0.066)
Cirrhosis: PHES score >0 (30)	0.138±0.002$^{\wedge}$ (0.134-0.142)	0.106±0.002 (0.101-0.111)	0.134±0.006 (0.123-0.145)	0.067±0.001 (0.064-0.070)
Cirrhosis: PHES score 0 to -4 (47)	0.138±0.002$^{\wedge}$ (0.135-0.142)	0.106±0.002 (0.102-0.110)	0.132±0.004$^{\wedge}$ (0.123-0.141)	0.063±0.001$^{\wedge\wedge\wedge *}$ (0.061-0.065)
Cirrhosis; PHES score -4 to -8 (16)	0.133±0.003$^{\wedge\wedge}$ (0.127-0.139)	0.111±0.003 (0.104-0.117)	0.127±0.008$^{\wedge}$ (0.112-0.142)	0.065±0.002$^{\wedge}$ (0.061-0.069)
Cirrhosis: PHES score <-8 (13)	0.139±0.003 (0.132-0.145)	0.130±0.004$^{\wedge\wedge\wedge ** \# \# \dagger\dagger}$ (0.122-0.137)	0.105±0.009$^{\wedge\wedge\wedge ** \#}$ (0.088-0.122)	0.059±0.002$^{\wedge\wedge\wedge ** \dagger}$ (0.055-0.064)

Data are expressed as estimates from linear mixed models ± SEM (95% CI)

PHES Psychometric Hepatic Encephalopathy Score

Significance of the difference between the reference population and the various patient subgroups; ^ $p<0.05$; ^^ $p<0.01$; ^^^ $p<0.001$
Significance of the difference between patients with PHES score (>0) and the other patient subgroups; * $p<0.05$; ** $p<0.01$; *** $p<0.001$
Significance of the difference between patients with PHES score (0 to -4) and the other patient subgroups; $^{\#}$ $p<0.01$; $^{\#\#}$ $p<0.001$
Significance of the difference between patients with PHES score (-4 to -8) and the other patient subgroups; $^{\dagger}p<0.05$; †† $p<0.001$