Event-related cerebral potentials for the diagnosis of subclinical hepatic encephalopathy in patients with liver cirrhosis

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Abstract

Purpose: Subclinical hepatic encephalopathy (SHE) seems to be a common problem in liver cirrhosis, however, studies assessing the pathogenesis of this disease remain unclear.

Currently no gold standard exists for the diagnosis of this complex neuropsychiatric syndrome. The present study was undertaken firstly to examine the diagnostic usefulness of auditory event-related cerebral potentials (ERPs) in the detection of SHE, and secondly to compare it with that of the most validated psychometric test.

Material and methods: 22 patients with liver cirrhosis without overt hepatic encephalopathy and 28 healthy controls were studied, using auditory ERPs. In addition they underwent a battery of neuropsychological and laboratory tests.

Results: P300 latency analysis turned out that cirrhotics patients had significantly longer P300 latency than controls. The only neuropsychological test showing significant difference between clinical and control group was the similarities subtest of WAIS-R.

Conclusions: The results of the present study suggest that ERPs are more sensitive method than psychometric tests in detecting early changes in the brain function of patients with cirrhosis and for this reason this neurophysiological method should be applied in clinical practice.

Key words: subclinical hepatic encephalopathy, neuropsychological tests, event-related cerebral potentials (ERPs).

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Introduction

Subclinical hepatic encephalopathy (SHE) has been defined as a condition in which patient with cirrhosis, regardless of its etiology, demonstrate a number of quantifiable neuropsychological defects, yet, on clinical examination have a normal mental and neurogical status [1]. The prevalence of SHE has been reported to vary from 30% to 84%, depending on the tests and population used [2]. Recently, several investigators have used neurophysiological tools such as quantitative electroencephalogram (EEG) analysis and exogenous or endogenous evoked potentials (EP) for the diagnosis of SHE [3]. The use of exogenous EP failed to detect SHE with an accurate sensitivity. However, the use of endogenous event-related cerebral potentials (ERPs) seems to be really helpful in diagnosis of SHE [4,5].

The P300 complex of visual or auditory event-related potentials appears to be frequently and consistently abnormal in cirrhotic patients with subclinical encephalopathy, in contrast to early components N100 and P200 which do not differentiate between SHE group and controls [3]. The P300 latency, which is reported to reflect the duration of evaluation process for an event or a stimulus, is significantly prolonged in cirrhotic patients than in controls. In the recent auditory ERPs study of Saxena et al. the mean P300 latency came to 363.5 msec in cirrhotic patients with SHE versus 349.3 msec in controls [6]. In addition, the mean P300 latency of cirrhotic patients, who were more than 40 years of age were significantly prolonged (369.9 msec) when compared with the latency of the younger cirrhotic patients (351.9 msec). Similarly, in the visual ERPs study of Giger-Mateeva et al. P300 latency of some cirrhotic patients without overt encephalopathy significantly exceeded the corresponding mean for controls [7]. Jones et al. have found in the visual ERPs study that the prolongation of P300 latency in cirrhotic patients without overt HE may occur in the absence of abnormalities of a standard psychometric test [8]. This neurophysiological finding discloses the existence of a delay of the stimulus evaluation time in cirrhosis even without

overt hepatic encephalopathy. Some investigators suppose that the P300 latency prolongation can originate from structural abnormalities of astrocytes known from Alzheimer's disease [9]. Others suggest that these abnormalities reflect metabolic neuropathophysiological, rather than structural, neuronal changes. The reversibility of at least some prolonged latencies of P300 is consistent with this concept [8,10].

However, controversy exists in the literature whether this neurophysiological method is as sensitive as psychometric tests. In the recent auditory ERPs study of Senzolo et al. P300 analysis did not prove very useful in discriminating patients with SHE [11]. Similarly, in the auditory ERPs study of Amodio et al., P300 latency provided little additional information for diagnosis of subclinical hepatic encephalopathy [12]. The present study was therefore undertaken firstly to examine the diagnostic usefulness of auditory P300 in the detection of SHE, and secondly to compare it with that of the most validated psychometric test.

Material and methods

For the experiment, 22 patients with a diagnosis of chronic cirrhosis were selected (11 women and 11 men). They all had no symptoms of focal damage to the central and peripheral nervous system and had no subjective feeling of lack of any deficits in the nervous system.

They were undergoing treatment in the University Hospital of Jagiellonian University in Cracow. The aetiology of cirrhosis was based on: clinical features, laboratory tests (blood plasma measures of: AST, ALT, albumines, prothrombin time, bilirubin, ammonia), serological tests of HBsAG, anti HCV antibodies, autoimmune markers as appropriate, history of alcohol and drugs abuse, genetic tests for haemochromatosis, ultrasonography to measure liver span, echo parenchyma and portal vein diameter, to confirm portal hypertension and ascites, endoscopy to check for presence of esophageal varices, where possible (no coagulopathy) liver biopsy for histopathological assessment.

Exclusion criteria from the clinical group were encephalopathy symptoms or signs of pathology observed in neurological assessment. Ages in the clinical group ranged from 27 to 59 (mean=48.7 years, SD=10.5). All the subjects were volunteers and were asked to participate in the experiment by the doctor taking care of them (participation was not enforced in any way and about 2/3 of patients agreed to participate).

Beside the clinical group 28 healthy subjects (20 women and 8 men) were included into the study as the controls. Their age was matched to the clinical group (mean=45.7; SD=10.9). All of them reported being free of neurological and psychiatric disorders.

All the subjects passed a battery of neuropsychological tests to assess for any cognitive deficits.

Nuclear Magnetic Resonance

All subjects were examined in TSE T1, TSE T2 and FLAIR sequence in transverse and sagittal planes with slices 3 mm and 5 mm thick. It revealed no focal damage to the CNS.

Neuropsychological Tests

To assess for any cognitive deficits, a battery of neuropsychological tests was used. They included (in order of applying): tapping test – right hand, tapping test – left hand (mean of 5 trials), memory test – spontaneous recall of 2 logical texts (8 and 16 positive elements) with recall of the first text afterwards (distraction), one-minute trials of verbal fluency: 2 semantic categories (animals and plants), 2 phonological categories (Polish nouns beginning with 'k' and 's' – chosen because of the highest noun frequency in Polish), Colour and Shape Sorting Test (the result of 2 categories found was treated as correct), Trail Making Test A+B (time of execution was analyzed), WAIS-R (Polish version), Beck Depression Inventory (score below 12 was interpreted as lack of depression symptoms). All the tests were run in a single session, which lasted approximately 2 hours.

Results of all tests and statistical significance of the difference between control and clinical group are depicted in *Tab. 1*. The only test showing significant difference between clinical and control group was the similarities subtest of WAIS-R test.

EEG recording and analysis

Scalp voltages were collected from Ag/AgCl active electrodes (with pre-amplifiers) using the 32-channel BioSemi Active-Two system. Electrodes were secured in an elastic cap (Electro Cap International). The vertex electrode was used as reference. Afterwards, after the recording, the data were re-referenced to linked electrodes FT7, T7, TP7, FT8, T8, TP8. The vertical electrooculogram (EOG) was recorded from electrodes placed above and below the right eye. The horizontal EOG was recorded from electrodes positioned at the outer canthus of each eye. The electrical signals were digitized within a sampling rate of 512 Hz. A digital bandpass filter set from 0.1 to 40 Hz (attenuation – 24 dB) was applied to all the data prior to running analyses, in order to reduce high frequency content, irrelevant to the components of interest. Additionally notch filter (50 Hz) was applied to the data.

Procedure

The experimental session took place in a dimly-lit sound-attenuated chamber. The participants were seated in a comfortable chair and presented a series of auditory stimuli. They all followed the odd-ball paradigm [13]. Two types of auditory stimuli were presented to them: "standards" (2 kHz, 85% chance of appearance), and "deviants" (1 kHz, 15% chance of appearance) in a random order. Each tone lasted for 100 milliseconds (rising/falling time 10 ms), and was presented at the level of 60 dB. The interstimulus interval was random, within the range of 900-1100 milliseconds.

The experiment was run in two phases – "passive" and "active". During the passive phase subjects were instructed just to listen to the sounds and do nothing. During the "active" phase subjects were instructed to listen to the sounds and count the rare tones (the 15% minority of 1 kHz tones). Additionally subjects were instructed to try not to fall asleep and keep their eyes open, try to refrain from blinking and shaking their heads (in order to minimize electric artifacts).

Both parts of the experiment lasted until 80 (+/-5) deviant stimuli were presented (which usually lasted 10 minutes).

Table 1. **Comparison of results of neuropsychological tests between clinical and control group. Significant differences are written in bold letters**

	Clinical group		Control group		p	test used
	mean	SD	mean	SD		
Tapping test						
right hand	45.1	9.2	43.8	7.6	0.38	
left hand	41.4	7.3	40.5	7.4	0.48	
Trail Making Test						
part A (time)	49.5	17.5	45.3	17.5	0.34	
part B (time)	102	49	90.7	39.8	0.25	
WAIS-R (PL)						
general IQ	98.2	103.0	13.9	18.0	0.33	
verbal	105.2	109.0	13.8	17.8	0.43	
information	10.2	10.5	2.3	3.1	0.77	
digit span	8.4	8.8	2.6	3.4	0.64	
vocabulary	13.2	13.5	2.4	2.6	0.67	
arithmetic	10.2	11.3	2.6	3.0	0.19	Student-t Test
comprehension	12.2	12.2	2.7	2.7	0.98	
similarities	**9.1**	**10.9**	**2.6**	**2.8**	**0.03**	
performance	91.3	97.5	12.7	13.3	0.12	
picture completion	9.1	10.4	2.9	2.9	0.12	
picture arrangement	7.5	8.7	2.0	2.3	0.07	
block design	8.3	9.5	2.4	2.6	0.14	
digit symbol	8.1	9.2	2.8	2.5	0.16	
object assembly	8.8	9.0	2.3	2.2	0.86	
Memory test						
text I	7.3	1.0	7.3	0.9	0.95	
text II	9.6	3.6	8.7	3.6	0.42	
text I (distraction)	5.6	2.9	5.6	2.8	0.95	
	N	**%**	**N**	**%**		
Verbal fluency						
animals	23.5	7.1	22.1	6.4	0.49	
plants	21.3	6.3	19.0	6.6	0.26	
letter ‘k’	11.4	5.6	12.9	5.6	0.38	chi-square Test
letter ‘s’	12.1	4.1	11.2	4.3	0.49	
Sorting (2 categories found)	18	95	23	88	0.47	
Beck Depression Inventory (score <12)	8	42	13	48	0.69	

Between the phases subjects were given opportunity to rest for 5 minutes. The “passive” phase was included only in order to calibrate and validate the hardware, and its results are not included into this work (moreover, its results did not differentiate the two groups).

Whole procedure lasted approximately 25 minutes for both parts not including the time needed for electrodes placement.

Data analyses

Continuous EEG recording was divided within each subject into 1 second epochs, time-fixed to the onset of the target tones (deviant stimuli). Each epoch started 100 msec before the onset of the stimuli, and ended 900 msec after. After that those 1000 msec epochs were averaged into one epoch, giving so-called event-related potential.

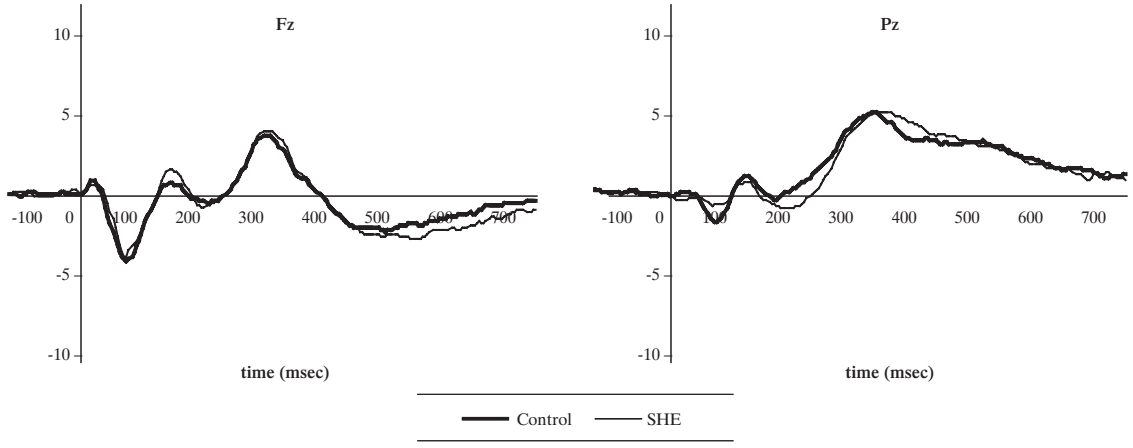
Baseline correction was performed using the average EEG activity in the 100 msec preceding the onset of the target tone as a reference signal value. Following baseline correction ocular correction was performed using Gratton and Coles algorithm [14].

After that, trials containing artifacts on electrodes of interest (Fz, Pz, and the reference electrodes) were rejected. The criteria were: amplitude beyond +/- 100 μV; change of amplitude within one trial greater than 150 μV, steepness of change greater than 35 μV per msec. Less than 15% of the trials were excluded by this operation.

The P300 component was defined as the largest positive-going peak occurring as a reaction for the deviant stimuli within a specific latency window: 250-450 msec. Peak amplitude was measured relative to the prestimulus baseline (100 ms), and peak latency was measured from the time of stimulus onset.

In addition to statistical tests, percent of abnormal P300 latency results was reported. The criterion for qualifying scores as normal or abnormal was taken from Policht et al. [15]. It defines formula for computing Z-score for P300 latency taking age under consideration ($Z = [\text{actual value}-(250+1.4*\text{age})]/40$). Values of $Z>2$ were considered abnormal.

Figure 1. **Event-related potentials for deviant stimuli in odd-ball paradigm on electrodes Fz and Pz – comparison of control and clinical groups**



Results

Fig. 1 depicts grand averages (averages of event-related potentials for all subjects within each experimental group) for the deviant stimuli from clinical and control groups for two electrodes position: Fz and Pz.

Two characteristics of the P300 component were compared – its amplitude and latency (their definition is described in Data Analyses section). Two Student's T-tests were run. For P300 amplitudes there was no significant difference between control and clinical groups (means 7.72 and 7.75 accordingly). P300 latency analysis turned out that cirrhotics had significantly longer P300 latency than controls – on average by 22 ms (395 and 373 accordingly; $t=2.35$; $p<0.05$).

Beside that, P300 was correlated with demographic and neuropsychological variables, to see if they influenced P300 latency on whole population (controls + cirrhotics). It turned out that there were two other variables significantly linearly influencing the latency: age ($r=0.39$; $p<0.01$) and the result in Beck Inventory ($r=0.41$; $p<0.01$).

To see if any of these variables explained variance of any other, P300-correlated variable, all three variables (group, age and Beck result) were entered into linear hierarchical regression analysis. Beck result was entered as the first variable, then group, and then the age. At the end only Beck's and group's betas were significant (Beck beta=0.331; group beta=0.31) and age didn't exceed significance threshold (beta=0.23). It turned out that the age had some common variance with the result of Beck inventory ($r=0.39$; $p<0.05$). Whole model of regression was significant ($R^2=0.28$; $p<0.01$).

Additionally, further correlations were looked for inside the clinical group only, between the P300 latency and the biochemical variables. It turned out that level of ALAT enzyme was significantly correlated with P300 latency ($r=-0.51$; $p<0.05$).

Beside the statistical analysis, standard count of normal/ /abnormal P300 latency was performed. It turned out that among the clinical group there were 8 subjects which qualified as abnormal (36%); in the control group there were 7 subjects with abnormal P300 latency (25%).

Discussion

The present study has demonstrated that latency of P300 component of ERPs are significantly prolonged in cirrhotic patients without overt encephalopathy compared with P300 latency in controls. Cirrhotic patients with prolonged latency of P300 may have subclinical hepatic encephalopathy. Our findings complement and extend previous reports on event-related cerebral potentials in patients with liver disease. The ERPs studies have been shown to be of great value in detecting latent stages of encephalopathy [6-8]. As opposed to conclusion of some investigators (Amodio et al., Senzolo et al.) we have demonstrated that P300 latency can provide useful information for diagnosis of cirrhotic patients in clinical practice [11,12].

An important finding of our study is that ERPs performed better than any of the neuropsychological screening tests used in detection of SHE. The event-related potentials seem to be more sensitive method than psychometric tests in detecting early changes in the brain function of patients with cirrhosis and for this reason this method should be applied in the assessment of them.

It is generally accepted that a lot of factors like age or severity of liver disease could influence the neurophysiological data and for this reason they were included in regression analysis. Results revealed that among the various factors in our model, only two had significant importance: group (patients vs controls) and depression symptoms. Many studies of brain function have suggested that depression is associated with cerebral hypoactivity. The latency of P300 component was found to be significantly prolonged in cases of major depression as compared to that of controls [16,17]. The results of our study are consistent with these findings. However, further investigations are required to

elucidate the diagnostic and predictive role of latency of P300 in the cases of depression. What is the most important, this result do not minimize the influence of liver disease on prolongation of P300 latency in our study. In addition, P300 latency prolongation has appeared as independent of aging. It means that age factor may be less important in neurophysiological diagnosis of SHE than some investigators has suggested [12].

It is only important to remember to control for all possible confounds of latency while matching patients in the control group. Age seems to be one such factor, depression level seems to be another. To our knowledge there is no study on P300 latency and SHE which controlled for depression. It is especially important when subjects are divided into normal and abnormal P300 latency groups. The rule of thumb was to qualify as abnormals subjects whose latency was 2 standard deviations off the norm. Recently what is norm for P300 latency has been adjusted for age factor [12]. It may be also important to take depression under consideration, especially that it showed stronger influence than age.

The present study has demonstrated that neurophysiological tests can add valuable information in the assessment of early stages of encephalopathy compared to the test battery and they should be used in the diagnosis of this neuropsychiatric syndrome. The auditory event-related potentials represent a promising tool for the objective diagnosis of subclinical hepatic encephalopathy for many reasons. Firstly, this method is reproducible, relatively easy to perform, non-invasive and safe to implement as compared to the other brain structural or metabolic studies. Secondly, it is more sensitive for the diagnosis of SHE than the established psychometric tests. Thirdly, it is not influenced by learning effects by the patient. We conclude that auditory ERPs are strongly recommended for diagnosis of subclinical hepatic encephalopathy.

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