Visual event-related components in human. A diagnostic tool for early detection of metabolic brain disorder
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CHAPTER V

THE EFFECT OF FLUMAZENIL ON VISUAL EVENT-RELATED POTENTIALS IN CLINICALLY NON-ENCEPHALOPATHIC PATIENTS WITH CIRRHOSIS


Abstract
The effects of flumazenil on the latencies and amplitudes of the visual event-related potentials (ERPs), number-connection test (NCT) and visual and auditory reaction times (VRT and ART) were evaluated in 10 patients with cirrhosis without clinical evidence of hepatic encephalopathy (HE). Delayed latencies of the ERP component P3a and/or P3b were found in three patients and prolonged NCT results were found in two other patients. However, changes in the latencies and amplitudes of the ERP components (N200, P3a, and P3b) during 40 minutes following infusion of flumazenil (1 mg) and placebo were similar. Results of the three psychometric tests were not abnormal in any of the patients studied and did not change significantly after either flumazenil or placebo infusion. Eight of the 10 patients felt more alert for several minutes after the administration of flumazenil, whereas no patient experienced any change of perception after infusion of placebo. While the findings suggest that prolongation of the latencies of P3a and P3b may be a component of the syndrome of subclinical HE, they provide no support for the hypothesis that these neuro-electrophysiological abnormalities in cirrhotic patients are attributable to increased brain levels of natural benzodiazepines (BZs).

Introduction
Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome that complicates both acute and chronic hepatocellular failure; it is generally considered to be reversible and to have a multifactorial pathogenesis (Basile et al., 1991). In the earliest clinical stage (stage I) subtle personality changes, discrete psychomotor dysfunction and alterations of sleep rhythm may occur. Subclinical HE (SHE) is recognized as an entity in patients with cirrhosis (chronic hepatocellular disease, Gitlin et al., 1986). The term SHE has been applied in cirrhotic patients with abnormal results of psychomotor tests and/or impaired neuro-electrophysiological function, or both, in the presence of normal results of a routine clinical neurological examination (Giger-Mateeva et al., 1999b, chapter 4 of this thesis; Jones et al., 1990; Kügler et al., 1992). The prevalence of SHE in cirrhotic patients without overt encephalopathy is uncertain, although a serious attempt to clarify this issue has
recently been made (Kügler et al., 1992). The subpopulation of cirrhotic patients with SHE or stage I HE may superficially appear to have normal mental function, particularly verbal function. However, impaired mental function may be demonstrated in these patients by finding subnormal psychometric test results and/or prolonged latencies of visual and auditory event-related potentials (N250, P300; Davies et al., 1990; Kügler et al., 1992). Recently we have reported on significantly altered latencies of the visual event-related P3a and P3b potentials in cirrhotic patients without overt HE (Giger-Mateeva et al., 1999b, chapter 4 of this thesis). Evidence has been accumulating which suggests that endogenous benzodiazepine receptor ligands (i.e., natural benzodiazepines, BZs) with agonist properties may contribute to the manifestation of HE (Basile et al., 1991; Mullen et al., 1988). Indirect evidence that increased levels of natural BZs might contribute to the manifestations of HE, is provided by reports on ameliorations of encephalopathy after the administration of BZ antagonists to animal models of fulminant (i.e., acute) hepatic failure (Basset et al., 1987; Basile et al., 1991) and in humans with cirrhosis or acute liver failure (Bansky et al., 1989; Basile et al., 1991; Giger-Mateeva et al., 1999b) These reports raised the possibility that ameliorations of HE may be induced by displacing natural BZ agonist ligands from central BZ receptors. Flumazenil is a prototypic benzodiazepine antagonist which has been extensively characterised (Jones et al., 1990). Intravenous bolus injections of flumazenil have been reported to induce unequivocal (I-II stage) ameliorations of HE in about 60% of patients with overt HE due to cirrhosis (i.e., chronical liver failure) or acute liver failure (Basile et al., 1991). In addition, in a single extensively studied patient, the oral administration of flumazenil was associated with a complete remission of chronic intractable porto-systemic encephalopathy, including a return of dietary protein tolerance to normal (Ferenci et al., 1990). The effects of flumazenil on cirrhotic patients without overt HE or with stage I HE have not been systematically evaluated (Gooday et al., 1995). In this study consecutive cirrhotic patients without overt HE underwent a psychometric and neuro-electrophysiological evaluation. In these patients the effects of intravenously administered flumazenil on psychometric test results and latencies of evoked event-related components were investigated.

**Materials and methods**

Ten patients with histologically confirmed cirrhosis, without clinical evidence of HE, were evaluated electrophysiologically. The patients had normal visual acuity or visual acuity corrected to normal. There were two females and eight males, aged 40 to 60 years. The cause of cirrhosis was chronic viral hepatitis (seven patients) and alcohol abuse (three patients). At the time of the study no patient was known to be taking ethanol or any neuroactive drugs for at least the preceding three months, and none of these patients had any major medical disorder other than cirrhosis. In particular, none had Parkinson disease, Alzheimer disease, multiple sclerosis, diabetes mellitus, ophthalmological disorder or active infection. Any caffeine-containing beverages were prohibited for at least 12 hours before and during the
study period, since caffeine is known to stimulate the central nervous system (CNS), in particular mental alertness.

All patients were fully informed about the nature of the procedures and each of them signed a written informed consent form before participating in the study. The protocol of the study had been approved by the Medical Ethics Committee of the Academic Medical Centre, University of Amsterdam.

Patients were evaluated in a semi-darkened room (mean illuminance 30-40 lux). They sat at a distance of 1.20 m from a television screen on which two types of black-and-white checkerboard stimuli were presented. “Frequent” stimuli of 12’ checks were randomly replaced by “infrequent” 200’ stimuli (after every 4th to 8th appearance of 12’ checks). Both stimuli were presented at a low (10%) contrast and duration of 40 ms every 640 ms.

The recordings were derived from four Ag-AgCl scalp electrodes attached near Fz, Cz, Pz, and Oz positions. The EEG signal was sampled every 4 ms and filtered online between 0.6 and 4.5 Hz; artefacts were automatically rejected from the signal. The patient was asked to count the number of the presented events. At the end of each recording the reported number was compared to the number of events actually presented to evaluate the alertness of the patients.

Standard pattern onset/offset VEP to 200' checks at 10% contrast were also recorded (filter bandwidth: 0.6 and 4.5 Hz). Stimuli were presented for 40 ms every 640 ms.

The amplitudes of the evoked components were measured in relation to the mean voltage of the recording during the first 40 ms after the stimulus onset. The peak latencies of the components were measured from stimulus onset.

The number-connection test (NCT) was performed as follows: after explanation and demonstration, each patient was asked to connect in correct order as quickly as possible a sequence of numbers from 1 to 25, that were randomly distributed on a sheet of paper. The time (in seconds) to complete the test was recorded (Conn, 1977).

The reaction times to visual (red light; VRT) and auditory (beep; ART) stimuli were also evaluated. The patient was asked to press a button and release it as soon as a stimulus (visual or auditory) was displayed. The times to react to a stimulus (in ms) were recorded.

Blood samples for routine haematological and biochemical tests were obtained within three weeks of the psychometric and electrophysiological testing. Laboratory blood tests included serum bilirubin (total and direct), albumin, alkaline phosphatase, gamma-glutamyl transpeptidase (gamma-GT), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), prothrombin time and platelet count. Urine was obtained immediately before psychometric and electrophysiological evaluation, and assayed for benzodiazepines, alcohol and barbiturates (Abbott Laboratories, Abbott Park, IL). Alcohol was analysed using a Radiative Energy Attenuation method. Barbiturates were assayed using the “TDx/TDxFLx Barbiturates II U assay” (lower limit of detection 500 ng/ml) and benzodiazepines using the “TDx/TDxFLx”
Benzodiazepines assay" (lower limit of detection 200 ng/ml).

Normative data were established for subjects in the same age range (40 to 60 years). N200, P3a, and P3b, and the time to complete NCT were used to assess the neurophysiological status of the clinically non-encephalopathic cirrhotic patients. Patients were examined on two occasions: in the morning and in the afternoon of the same day. Each session started with a battery of psychometric tests (NCT, VRT and ART) and was followed by a neuro-electrophysiological assessment. During the latter, patients were randomized to receive an intravenous infusion of either placebo or flumazenil (1 mg) over a period of 2 min. The physicians conducting the neurophysiological experiments were "blinded" with respect to the content of the infusion.

Electrophysiological testing (to evoke N200, P3a, and P3b) was done at baseline (at the start of the evaluation), immediately before the injection, and every eight minutes after the injection for 40 minutes. The responses to 200' onset/offset checks (to assess P3a separately) were recorded twice: before and 20 min after the infusion. NCT was performed before and 10 min after the infusion. VRT and ART were determined before and 5, 10, and 20 min after the infusion. Immediately after the infusion the patient was requested to inform the investigator about any unusual sensation or perception. Each complete experimental session took about 50 minutes.

A repeat complete "blinded" experimental session was performed after a break of about four hours. If during the first session placebo had been infused, during the second session the drug (flumazenil) was infused, and vice versa. The psychometric and neurophysiological procedures performed during both sessions were the same.

Results

All patients were "well oriented with respect to time and place" and counted the events properly. Table 1 gives the mean event-related potentials (latencies and amplitudes) of the patients, evaluated before the intravenous infusion of either placebo or flumazenil. In five patients either the event-related potentials or the psychometric test results were abnormally delayed. The N200 component was
Table 1. Mean latencies and amplitudes of ERPs (of two successive recordings) in healthy subjects and cirrhotic patients without overt encephalopathy (age 40 to 60 years).

<table>
<thead>
<tr>
<th>ERPs</th>
<th>N200 latency (ms) at Oz</th>
<th>N200 amplitude (µV)</th>
<th>P3a latency (ms) at Fz</th>
<th>P3a amplitude (µV)</th>
<th>P3b latency (ms) at Pz</th>
<th>P3b amplitude (µV)</th>
<th>NCT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy mean ± SD</td>
<td>207±16 [18]</td>
<td>3.6±1.4</td>
<td>259±27 [18]</td>
<td>3.4±1.2</td>
<td>384±37 [20]</td>
<td>7.3±2.9</td>
<td>29±7 [20]</td>
</tr>
<tr>
<td>patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>-</td>
<td>320 **</td>
<td>5.7</td>
<td>504***</td>
<td>7.6</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>212</td>
<td>316 **</td>
<td>10.4</td>
<td>434*</td>
<td>13.2</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>186</td>
<td>270</td>
<td>3.3</td>
<td>398</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>210</td>
<td>256</td>
<td>7.4</td>
<td>412</td>
<td>7.6</td>
<td>44 ^^</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>400</td>
<td>3.9</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>228 *</td>
<td>4.8</td>
<td>288 *</td>
<td>4.4</td>
<td>420</td>
<td>4.0</td>
<td>47 ^^</td>
</tr>
<tr>
<td>7</td>
<td>220</td>
<td>1.3</td>
<td>240</td>
<td>1.9</td>
<td>346</td>
<td>2.7</td>
<td>43 ^</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>270</td>
<td>4.8</td>
<td>446 *</td>
<td>4.4</td>
<td>41 ^</td>
</tr>
<tr>
<td>9</td>
<td>198</td>
<td>1.1</td>
<td>340 **</td>
<td>8.4</td>
<td>440 *</td>
<td>8.8</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>190</td>
<td>1.1</td>
<td>262</td>
<td>6.4</td>
<td>454 *</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

(-) Patients, in whom no ERP could be detected
[n] Number of subjects/patients
SD, standard deviation of the mean

N200 latency (ms) | P3a latency (ms) | P3b latency (ms) | NCT (s) |
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<tr>
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</thead>
<tbody>
<tr>
<td>* &gt; mean±1SD (223)</td>
<td>* &gt; mean±1SD (286)</td>
<td>* &gt; mean±1SD (421)</td>
<td>^ &gt; 36</td>
</tr>
<tr>
<td>** &gt; mean±2SD (239)</td>
<td>** &gt; mean±2SD (313)</td>
<td>** &gt; mean±2SD (438)</td>
<td>^ ^ &gt; 43</td>
</tr>
<tr>
<td>*** &gt; mean±3SD (255)</td>
<td>*** &gt; mean±3SD (340)</td>
<td>*** &gt; mean±3SD (495)</td>
<td>^ ^ ^ &gt; 50</td>
</tr>
</tbody>
</table>

* indicates value greater than the corresponding mean value for healthy plus one SD, in particular, greater than the calculated number given in parenthesis
** indicates value greater than the corresponding mean value for healthy plus three SD, in particular, greater than the calculated number given in parenthesis
detected (at Oz) in seven patients (10 tested) and its latency was consistently within normal limits. The P3a component was detected (at Fz) in nine patients and in three of them (patients 1, 2 and 9) its latency was abnormally delayed (i.e., greater than the mean latency + 2SD (standard deviation) of the mean of 20 healthy subjects aged between 40 and 60 years). The P3b component was detected (at Pz) in all the patients and was delayed in one (patient 1).

In two patients (patients 4 and 6) the time to complete the NCT was abnormal i.e., greater than 43 s, (the mean time + 2SD of the mean of the age-matched control subjects). In these two patients the neurophysiological test results were within normal limits.

Fig. 1 shows the latency variations of P3a and P3b components in the patients with delayed P3a and/or P3b components (patients 1, 2 and 9) and delayed NCT tests (patients 4 and 6). No significant changes were found in the latency of P3a with time after the administration of placebo or flumazenil. In the patient in whom both P3a and P3b were delayed (patient 1), changes in the latency of P3b with time after the administration of placebo and flumazenil were not significantly different. The amplitudes of P3a and P3b did not change significantly in time.

There were no appreciable differences between the time needed to complete a NCT before (at 0 min) and after administration of placebo or flumazenil. Also corresponding times for the VRT and ART tests before and after administration of placebo or flumazenil were not significantly different (Fig. 2).
placebo

flumazenil

P3a latency (ms)

P3a latency (ms)

P3b latency (ms)

P3b latency (ms)

time (min)

time (min)

1 2 4 6 9
Results of routine serum biochemical liver tests, prothrombin time and platelet counts were consistent with the presence of cirrhosis (data not shown). In all 10 patients the urine samples were negative for alcohol, benzodiazepines and barbiturates. The Child-Pugh Scores/Grades of the patients are: pat. 1: 5/A, pat. 2: 8/B, pat. 3: 6/A, pat. 4: 9/B, pat. 5: 5/A, pat. 6: 8/B, pat. 7: 5/A, pat. 8: 5/A, pat. 9: 5/A, pat. 10: 6/A. (Scores range from 5 to 15, with 5 best and 15 worst; grade A is better than B, which is better than C).

In eight of the 10 patients the administration of flumazenil was followed within minutes by a feeling of being more alert, awake and/or concentrated. One patient reported feeling rather drowsy immediately after the infusion of flumazenil. The remaining patient reported no change in perception after flumazenil administration. In all 10 patients no changes in perception were reported after placebo infusion.

**Discussion**

The present investigation shows that in five of 10 clinically non-encephalopathic cirrhotic patients psychometric and/or neurophysiological tests were abnormal. These findings suggest that in half of the patients studied subclinical hepatic encephalopathy (SHE) may have been present (Giger-Mateeva et al., 1999b, chapter 4).

In the three patients with abnormal event-related test results, flumazenil did not correct the prolonged latencies of P3a and/or P3b and did not improve psychometric test results.

In contrast, subjectively in eight of the 10 patients, infusion of flumazenil was followed by an apparent stimulation of the CNS, consistent with a decrease in GABA-mediated neurotransmission due to displacement of natural BZs from central BZ receptors (Higgit et al., 1986). The feeling of drowsiness in another patient immediately after the infusion of flumazenil may be attributable to the partial agonist intrinsic activity of flumazenil (Basile et al., 1991). Abnormalities of psychometric tests and neuro-electrophysiology can be detected in appreciable proportion of patients with cirrhosis, impaired hepatocellular function, and no symptoms or signs of HE on routine neurological exam. Cirrhotic patients with such abnormalities may be considered to have SHE (Gallai et al., 1995; Giger-Mateeva et al., 1999b; Kügler et al., 1992; Quero et al., 1996; Van der Rijt et al., 1984). Some of the abnormalities of psychometric tests and neuro-electrophysiology in such patients can be reversed by flumazenil (Ferenci et al., 1996; Gooday et al., 1995), implying that increased brain levels of natural BZs contribute to them (Basile et al., 1991).
Fig. 2 Scatter diagram of the ART (A), VRT (B) and NCT (C) with time (min) after the administration of placebo and flumazenil. The corresponding values before any administration are plotted at zero time. Paired t-test results between the values before and after flumazenil are summarized in the table (bottom right, D). The number in parenthesis gives the number of patients, t is the distribution and P is the two-tailed probability.

Other abnormalities of neurophysiology, such as prolonged latencies of P3a and/or P3b, do not appear to be reversed by flumazenil, and hence may not be mediated by increased brain levels of natural BZs. Indeed, factors other than increased brain levels of natural BZs may contribute to the syndrome of subclinical HE. Reversal of the neurological effects of normal or increased brain levels of natural BZs by flumazenil may account for transient increased alertness in cirrhotic patients following infusion of flumazenil (Basile et al., 1991).