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Research report

Abnormal cortical synaptic plasticity in minimal hepatic encephalopathy

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ABSTRACT

Minimal hepatic encephalopathy (MHE) represents the earliest stage of hepatic encephalopathy (HE). MHE is characterized by cognitive function impairment in the domains of attention, vigilance and integrative function, while obvious clinical manifestations are lacking. In the present study, we aimed at assessing whether subjects with MHE showed alterations in synaptic plasticity within the motor cortex. Previous findings suggest that learning in human motor cortex occurs through long-term potentiation (LTP)-like mechanisms. We employed therefore the paired associative stimulation (PAS) protocol by transcranial magnetic stimulation (TMS), which is able to induce LTP-like effects in the motor cortex of normal subjects. Fifteen patients with MHE and 15 age- and sex-matched cirrhotic patients without MHE were recruited.

PAS consisted of 180 electrical stimuli of the right median nerve paired with a single TMS over the hotspot of right abductor pollicis brevis (APB) at an ISI of 25 ms (PAS25). We measured motor evoked potentials (MEPs) before and after each intervention for up to 30 min. In healthy subjects the PAS25 protocol was followed by a significant increase of the MEP amplitude. On the contrary, in patients with MHE the MEP amplitude was slightly reduced after PAS.

These findings demonstrated that associative sensorimotor plasticity, an indirect probe for motor learning, is impaired in MHE patients.

1. Introduction

It is well known that 30% to 45% of patients with liver cirrhosis develop a spectrum of potentially reversible neurocognitive deficits, termed 'hepatic encephalopathy' (HE) (Poordad, 2007; Stinton and Jayakumar, 2013; Vilstrup et al., 2014). Minimal hepatic encephalopathy (MHE) represents the earliest stage of HE (Amodio et al., 2004; Dharel and Bajaj, 2015; Morgan et al., 2015). It frequently remains undiagnosed, partly due to the time consuming neuropsychological and neurophysiological examinations which require highly specialized personnel and specific testing equipment. The diagnosis of MHE may also be overlooked because verbal functioning is usually preserved while the cognitive deficits predominantly involve the areas of overall performance and psychomotor activities. On the other hand, the term 'MHE' may be misleading. It may suggest that the clinical impact of HE at this very early stage is rather limited. However, MHE represents a considerable medical problem, with significant clinical implications for the patient as well as public safety concerns.

It is now possible to study the motor cortical plasticity by using an experimental intervention known as paired associative stimulation (PAS) (Stefan et al., 2000). The physiological and pharmacological profile of these modifications in primary motor cortex (M1) excitability suggests that a long-term potentiation (LTP)-like mechanism may underlie the PAS induced synaptic plasticity (Stefan et al., 2002). LTP is a long-lasting enhancement of synaptic transmission efficacy and is considered the basis for some forms of learning and memory.

When a repetitive electrical stimulus to the median nerve is paired with a transcranial magnetic stimulation (TMS) pulse over the contralateral motor cortex with an interstimulus interval (ISI)

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Table 1
Demographic and clinical characteristics of the patients with minimal hepatic encephalopathy and the control subjects.

	Patients	Controls
Age	58 ± 4	56 ± 2
Sex (M/F)	9/6	9/6
Child-Pugh Scale		
A	7	1
B	6	6
C	2	8
Venous blood ammonia (in μmol/l)	70.4 ± 26.4	
PHES	−0.57 ± 1	−0.13 ± 1
CFF	40 ± 5	48 ± 2

PHES = psychometric hepatic encephalopathy score; CFF = critical flicker frequency.

of 21.5–25 ms, a LTP-like synaptic plasticity is induced in the corticospinal system.

Aim of this study was to investigate the motor cortex LTP-like synaptic plasticity by means of PAS in patients with MHE.

We hypothesize that motor cortex LTP-like synaptic plasticity is impaired in MHE patients. Therefore, we chose to employ PAS to examine LTP-like phenomena in the sensorimotor cortex of patients with MHE.

2. Materials and methods

2.1. Patients

Fifteen patients with hepatic cirrhosis (HC) and MHE and 15 age-matched cirrhotic patients without MHE were enrolled in the study after signing an informed consent. Clinical and demographic characteristics of the patients are shown in Table 1. Physical examination, standard laboratory tests, and a standardized battery of psychometric tests aimed at detecting MHE were administered to all participants. The diagnosis of HC was based on clinical, biochemical, and ultrasonographic data. Since similar studies in this patient population are lacking, and therefore the statistical power is difficult to be determined, we chosen to adopt in this preliminary study a convenience sample. On the other hand, many of the initially selected patients were not enrolled in the study due to the exclusion criteria. These were: contraindication to TMS; overt HE or history of overt HE, infection, recent (<6 weeks) antibiotic use or gastrointestinal bleeding, history of recent (<6 weeks) use of drugs affecting cognitive function as well as cortical excitability (e.g., benzodiazepines, anti-epileptic and/or psychotropic drugs), history of shunt surgery or transjugular intrahepatic portosystemic shunt for portal hypertension, electrolyte imbalance, renal impairment (serum creatinine > 1.5 mg/dL), presence of hepatocellular carcinoma, or severe medical problems (e.g., congestive heart failure, pulmonary disease, other neurological or psychiatric disorders).

Neuropsychological assessment and Critical flicker frequency (CFF) test (Sharma et al., 2000; Kircheis et al., 2002) were carried out in each participant on the same day. Patients with HC were classified with or without MHE by using the Italian validated version of the Psychometric Hepatic Encephalopathy Score (PHES) (Amodio et al., 2008). This battery comprises 5 psychometric tests: the digit symbol test, the number connection test A, the number connection test B, the serial dotting test, and the line tracing test. Overall, this neuropsychological battery examines motor speed and accuracy, visual perception, visuospatial orientation, visual construction, concentration, attention, and working memory. PHES individual results were summarised in a sum-score ($PHES_{TOTAL}$) and HE patients with a $PHES_{TOTAL}$ equal to or lower than −4 were included in the MHE group. Visual persistence, defined as the capability of the visual system to react to changes in the visual field, was measured with the CFF test. This test, broadly used for the diagnosis of MHE, has shown higher sensitivity and specificity when compared with other psychometric tests devoted to this purpose. CFF measurements were obtained in a quiet, semi-darkened room without distracting noises by using a portable, battery-powered analyzer.

2.2. Experimental design

All subjects were seated in a comfortable reclining chair during the experiment. Both arms were supported by a pillow. Resting motor threshold (RMT) and motor-evoked potentials (MEPs) were recorded from the right abductor pollicis brevis (APB) before PAS (baseline), immediately (T0), 15 min (T15) and 30 min (T30) after the end of PAS on the dominant side of the MHE patients and the cirrhotic patients without MHE (controls). All TMS examinations were carried out in the early afternoon, at 3:00 p.m.; in fact, PAS is more effective in subjects tested in the afternoon than in the morning, possibly because diurnal rhythms, involving neuromodulators such as melatonin and cortisol, might also influence the effects of PAS (Sale et al., 2007).

2.3. Transcranial magnetic stimulation

Focal TMS was applied through a standard figure-of-eight coil with mean loop diameters of 9 cm connected to a High Power Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). The coil was placed tangentially to the scalp with the handle pointing backwards and laterally at a 45° angle to the sagittal plane to induce a posterior–anterior current in the brain.

The site at which stimuli at slightly suprathreshold intensity produced the largest and most consistent MEP was chosen as the "motor hot spot" and used for TMS of the motor cortex.

2.4. Paired associative stimulation

PAS protocol paired electrical stimulation of the median nerve with single-pulse TMS of the contralateral primary motor hand area.

PAS consisted of 180 electrical stimuli of the right median nerve at the wrist paired with a single TMS shock over the hotspot of right APB muscle at a rate of 0.2 Hz (Stefan et al., 2000, 2002). Electrical stimulation (square wave pulse; stimulus duration, 0.2 ms) was applied at an intensity of three times the perceptual threshold using a constant current generator (Digitimer, Welwyn Garden City, UK). TMS was applied at an intensity required to elicit a 1 mV MEP ($SI_{1\,mV}$). The effects of PAS given with an interstimulus interval of 25 ms between peripheral and TMS stimuli were tested (PAS25). Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived. The MEPs evoked in the APB were displayed online during the intervention to control for the correct coil position and stored for off-line analysis.

2.5. Surface electromyography and data analysis

EMG was recorded with Ag-AgCl surface electrodes from the right APB muscle using a belly-tendon montage. The signal was amplified and band pass filtered (32 Hz–1 KHz) by a Digitimer D-150 amplifier (Digitimer Ltd., Welwyn Garden City, Herts, UK) and was subsequently stored at a sampling rate of 10 KHz on a personal computer for off-line analysis (Signal Software, Cambridge Electronic Design, Cambridge, UK).

Single-pulse TMS was used to probe corticospinal excitability before and after PAS.