Clinical Neurophysiology 130 (2019) 911–916

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

GABA-ergic tone hypothesis in hepatic encephalopathy – Revisited

Stefan Jun Groiss [a,b,*], Markus Butz [a], Thomas J. Baumgarten [a], Nur-Deniz Füllenbach [c], Dieter Häussinger [c], Alfons Schnitzler [a,b]

[a] Institute of Clinical Neuroscience and Medical Psychology, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany
[b] Department of Neurology, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany
[c] Department of Gastroenterology, Hepatology and Infectiology, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany

ARTICLE INFO

Article history:
Accepted 22 March 2019
Available online 30 March 2019

Keywords:
Hepatic encephalopathy
GABA
TMS
Cortical excitability

HIGHLIGHTS

- GABA-ergic tone of the motor cortex was decreased in patients with hepatic encephalopathy (HE).
- Alteration of GABA-ergic neurotransmission in HE was disease severity dependent.
- Our results challenge the classical GABA hypothesis in HE.

ABSTRACT

Objective: The GABA hypothesis of hepatic encephalopathy (HE) proposes an increased cerebral GABA-ergic tone in HE but has not been investigated in vivo in HE-patients yet. Cortical GABA-ergic and glutamatergic neurotransmission in HE-patients were evaluated using transcranial magnetic stimulation.
Methods: Twenty-one patients with HE grade 1 and 2 and age matched controls participated in the study. GABA-ergic (short- and long-interval intracortical inhibition (SICI and LICI)) and glutamatergic (intracortical and short-interval intracortical facilitation (ICF and SICF)) excitability of the primary motor cortex (M1) and global corticospinal excitability (motor threshold, motor evoked potential recruitment curve (MEP-RC) were compared between the groups. SICI and ICF were correlated to the critical flicker frequency (CFF) as measure for disease severity.
Results: In HE-patients, the slope of MEP-RC was significantly shallower compared to healthy controls. SICI was significantly reduced in patients with HE grade 2 compared to healthy controls. In HE-patients, SICI and ICF was significantly correlated to CFF.
Conclusion: Although global corticospinal excitability was reduced in HE-patients, GABA-ergic inhibition was reduced in M1 depending on HE severity. Moreover CFF related alteration of GABAergic and glutamatergic neurotransmission in patients with HE could support the notion of a severity dependent alteration of cortical excitability.
Significance: The decrease of cortical GABA-ergic tone challenges the classical GABA hypothesis in HE.

1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric complication of chronic liver failure manifesting with ataxia, negative myoclonus and cognitive dysfunction, confusion or impaired consciousness (Häussinger and Blei, 2007). Based on the fact that the pattern of postsynaptic neuronal activity measured by visual evoked potentials in HE was similar to that induced by GABA-ergic drugs, it was proposed in 1982 that GABA-ergic tone is increased in HE. Over decades this concept has been widely accepted as the GABA hypothesis of HE (Schafer and Jones, 1982), although increasing experimental evidence, mostly from animal studies, suggest inconsistent GABA-ergic alterations depending on the type and grade of experimental HE and investigated brain region (Cauli et al., 2009). Transcranial magnetic stimulation (TMS) enables a non-invasive stimulation of the brain. Using paired-pulse techniques intracortical inhibitory and facilitatory mechanisms can be investigated (Paulus et al., 2013). In brief, short- and long-interval intracortical inhibition (SICI and LICI) are supposed to reflect $GABA_A$ and $GABA_B$ mediated inhibition,

* Corresponding author at: Department of Neurology, Heinrich-Heine University, Moorenstr. 5, 40225 Düsseldorf, Germany.
E-mail address: groiss-js@umin.net (S.J. Groiss).

https://doi.org/10.1016/j.clinph.2019.03.011
1388-2457/

respectively (Valls-Solé et al., 1992; Ziemann et al., 1996a; Hanajima et al., 1998; McDonnell et al., 2006), whereas intracortical and short-interval intracortical facilitation (ICF and SICF) are supposed to reflect glutamatergic facilitation (Tokimura et al., 1996; Ziemann et al., 1996b, 1998; Hanajima et al., 2002; Chen, 2004).

A recent study investigated cortical excitability in premanifest HE patients using paired pulse TMS and found increased GABA-ergic inhibition measured with SICI and reduced glutamatergic facilitation measured with ICF, which would be in line with the GABA hypothesis (Nardone et al., 2016). However, in patients with manifest HE, studies on cortical excitability are sparse and did not explicitly investigate GABA-ergic or glutamatergic neurotransmission (Nolano et al., 1997).

Here, we investigated cortical GABAergic and glutamatergic neurotransmission in detail in manifest HE patients with different disease severity and age-matched healthy control subjects using TMS.

2. Methods

2.1. Subjects

Twenty one patients with HE and ten age-matched healthy volunteers were recruited. Inclusion criteria were patients with manifest HE grade 1 and 2 according to the West-Haven Criteria, age over 18 years, understanding of the study protocol and given written informed consent. Patients with pacemakers or other metal implants, pregnancy, severe coagulopathy, alcohol consumption within the last 6 weeks, legal guardianship, further neurological or severe medical comorbidities were excluded from the study. Baseline characteristics are summarized in Table 1. All participants gave their prior written informed consent. The study was carried out in accordance with the declaration of Helsinki and was approved by the local ethics committee of the University Düsseldorf.

2.2. HE severity assessment

The HE severity assessment was performed based on the West-Haven criteria, psychometric testing and critical flicker frequency (CFF) (Kircheis et al., 2002, 2014). Details of psychometric testing has been described elsewhere (Kircheis et al., 2002, 2014). In brief, it consisted of a battery of 5 computer based neuropsychological tests with a total of 22 parameters investigating cognition, emotion, behavior and biologic regulation, that were chosen from the Vienna Test System (Dr. Schuhfried, Austria) (Kircheis et al., 2002, 2014). CFF is a simple, sensitive, and reliable way to quantify and monitor HE (Kircheis et al., 2002; Sharma et al., 2007). CFF was measured in a quiet, semi-darkened room without disturbing noises with a portable, battery-powered analyzer (Hepatonorm Analyzer; nevoLAB GmbH, Maierhöfen, Germany). The analyzer provoked an intrafoveal light stimulus with defined pulses of light. First, the analyzer was adjusted to generate a red light with a high frequency pulse (60 Hz) which gave the participant the impression of a steady light. Then, the frequency was decreased gradually until the participant had the impression that the steady light had changed to a flicker. The participants were instructed to register this change by pressing a hand-held switch. After repeating the process at least 5 times to ensure that the participant understood the procedure, another 10 trials were performed to calculate the mean and the standard deviation values for each participant (Romero-Gómez et al., 2007). Blood ammonia levels were also assessed. All investigations were assessed on the same day and prior to the TMS measurements.

2.3. Electromyographic recording

Electromyographic (EMG) activity was recorded from the right first dorsal interosseus (FDI) muscle using Ag-AgCl surface electrodes (9 mm diameter) in a belly tendon montage. The EMG signals were amplified (Digitimer D360, UK), band passed between 100 Hz and 5 kHz and digitized at a sampling rate of 5 kHz.

2.4. Transcranial magnetic stimulation

TMS was performed with a Magstim bistim magnetic stimulator (Magstim Co. Ltd, UK). The primary motor cortex with hotspot for the right FDI was stimulated with a figure-of-eight coil. The hotspot was defined as the area where we could provoke the largest MEP response in the FDI. Starting 5 cm lateral and 1 cm anterior to the vertex, the hotspot was defined by moving the coil in steps of 0.5 cm in anterior-posterior and medial-lateral directions. The coil was placed tangentially to the scalp, approximately perpendicular to the central sulcus, with the handle pointing backward and laterally at 45° from the midline, so that posterior-anterior current was induced in the brain. Active motor threshold (AMT) was defined as the lowest stimulation intensity that still evoked responses of approximately 100 μV during slight voluntary contraction of 10–20% which was monitored with an oscilloscope (Rossini et al., 2015). However, if a MEP response was followed by a clear silent period, smaller MEP of 50 μV were still considered as relevant response. We used this method for AMT definition to ensure that conditioning stimuli intensities for SICI are not too high and thereby corrupted by SICF (Peurala et al., 2008). Resting motor thresholds (RMT) was defined as the lowest stimulation intensity that still evoked responses of approximately 50 μV during complete relaxation of FDI (Rossini et al., 2015). The relative frequency method was used (Rossini et al., 2015). The subjects were asked to keep their right hand relaxed throughout the experiments and FDI activity was monitored in real-time with an oscilloscope. The order of experiments was counterbalanced across subjects.

Table 1
Baseline characteristics of HE patients and healthy controls.

	Controls	HE 1	HE 2	*p*
N (female/male)	10 (4/6)	11 (2/9)	10 (4/6)	
Etiology (alcoholic/other: viral or autoimmune hepatitis, NASH)	n. a.	8/3	8/2	
Age	60.5 ± 2.0	59.5 ± 3.3	63.3 ± 2.4	0.67
AMT (%MSO)	34.5 ± 1.2	37.8 ± 1.3	37.6 ± 2.3	0.32
RMT (%MSO)	48.5 ± 3.0	55.6 ± 3.1	49.3 ± 2.4	0.17
Test MEP size (mV)	0.63 ± 0.08	0.59 ± 0.08	0.61 ± 0.08	0.85
CFF (Hz)	40.2 ± 0.7	35.7 ± 0.7	31.8 ± 1.0	<0.000.1
Blood ammonia level (ng/ml)	n. a.	87.5 ± 12.9	65.9 ± 15.0	0.33

(NASH = non-alcoholic steatohepatitis, AMT = active motor threshold, RMT = resting motor threshold, % MSO = percentage of maximal stimulator output, Hz = Herz, n. a. = not applicable).

2.5. TMS parameters

2.5.1. Recruitment curve

The recruitment curve was measured using five individually adjusted stimulation intensities at 100%, 110%, 120%, 130% and 140% of RMT. Similar intensities have been used earlier (Chen et al., 1998; Hamada et al., 2008).

2.5.2. Paired-pulse paradigm

Four different measures, namely SICI, ICF, LICI and SICF, were studied.

SICI and ICF were studied with a subthreshold conditioning stimulus at 90% AMT preceding a suprathreshold test stimulus at ISIs of 2, 3, 4, 10 and 15 ms. For LICI measurements the test stimulus was preceded by a suprathreshold conditioning stimulus with an intensity of 110 % RMT and an ISI of 100 ms. For SICF the test stimulus was followed by a second stimulus at 110 % AMT with ISIs of 1.5 ms and 3 ms. Similar intensities and ISIs have been used earlier (Ziemann et al., 1996b; Hanajima et al., 1998; Hamada et al., 2008). Single test stimuli were applied as control conditions for size ratio calculation. Test stimulus intensities were adjusted individually to elicit MEPs around 0.5 mV when given alone. Ten trials were averaged for each condition. Each condition was applied in a shuffled order.

2.6. Statistical and data analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, CA, USA). Shapiro-Wilk test was used to test for normal distribution. Two-way repeated measures ANOVA was used to compare SICI-ICF (between subject factor (group); within subject factor (ISI): 2 ms, 3 ms, 4 ms, 10 ms, 15 ms), SICF (between subject factor (group); within subject factor (ISI): 1.5 ms, 3 ms) and recruitment curve (between subject factor (group); within subject factor (intensity): 100% RMT, 110% RMT, 120% RMT, 130% RMT, 140% RMT) between healthy controls and patients with HE 1 and HE 2, respectively. One-way ANOVA was used to compare LICI and baseline characteristics between the three groups except of blood ammonia levels, which were compared only between HE 1 and HE 2 patients with unpaired t-test. Post-hoc bonferroni tests were performed where applicable. Pearsońs correlation analyses were performed between both paired pulse measurements (SICI at 2 and 4 ms, ICF at 10 and 15 ms) and CFF and blood ammonia levels, respectively. For all statistical analyses, p-values were corrected for multiple comparisons. The level of significance was set to $p < 0.05$.

3. Results

Since normality was given, parametric tests were used for further analysis as described.

3.1. Baseline characteristics

Clinical demographic data and baseline TMS values did not differ between groups except for CFF (Table 1). Post-hoc analysis revealed significantly higher CFF values for healthy controls compared to HE 1 and HE 2 ($p < 0.01$ and $p < 0.001$, respectively) and for HE 1 compared to HE 2 ($p < 0.05$).

3.2. Recruitment curve

The slope of the recruitment curve was significantly shallower in patients with both HE 1 and HE 2 compared to healthy controls (significant effect on group: $F(3135) = 18.40$, $p < 0.0001$, significant effect on intensity: $F(4135) = 11.45$, $p < 0.0001$ and significant interaction group × intensity: $F(12,135) = 2.76$, $p < 0.002$). Post-hoc analysis revealed significantly smaller MEP sizes in HE 1 and HE 2 patients compared to healthy controls at 130 % RMT ($p < 0.0001$ each) and 140 % RMT ($p < 0.001$ each; Fig. 1A). No differences were found between HE 1 and HE 2.

3.3. Paired-pulse TMS

Significant differences of SICI/ICF curves were found between groups (significant effect on group: $F(2112) = 5.63$, $p = 0.0088$, ISI: $F(4112) = 25.85$, $p < 0.0001$ and significant interaction group - × ISI: $F(8112) = 2.11$, $p = 0.04$). Post-hoc analysis revealed a significantly reduced inhibition at ISIs of 2 ms and 4 ms for HE 2 patients ($p < 0.01$ each; Fig. 1B). SICF and LICI did not differ between groups (Supplementary Fig. S1). Correlation analysis demonstrated significant correlations between SICI 2 ms and CFF, SICI 4 ms and CFF as well as ICF 10 ms and CFF and ICF 15 ms and CFF (Fig. 2). No correlation was found between SICF and CFF or LICI and CFF. No correlation was found between TMS parameters and blood ammonia levels as well.

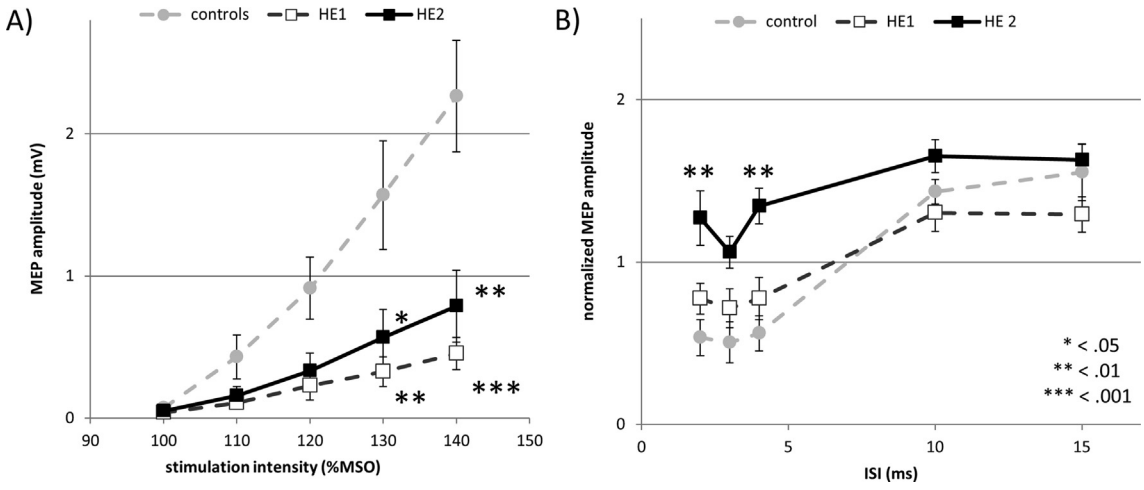

Fig. 1. (A) Recruitment curves of HE patients are significantly shallower compared to healthy controls. MEP sizes were significantly smaller in HE1 and HE2 patients compared to healthy controls at 130% RMT and 140% RMT. (B) SICI at 2 ms and 4 ms were significantly reduced for HE 2 patients compared to HE 1 patients and controls. (MEP = motor evoked potentials, RMT = resting motor threshold, SICI = short-interval intracortical inhibition).

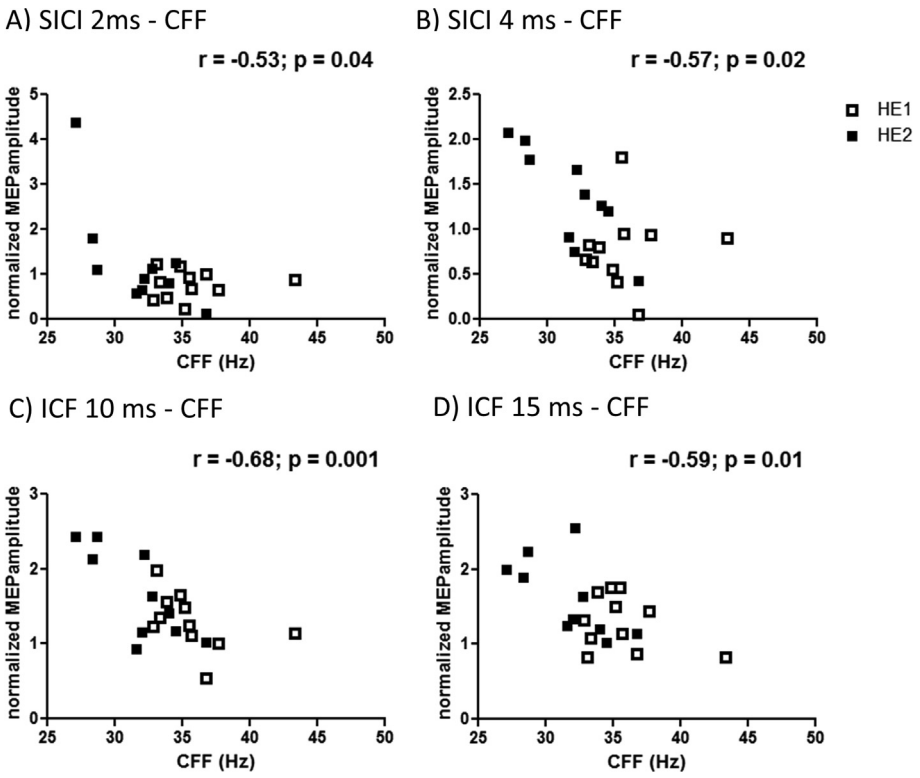

Fig. 2. Correlation analyses between SICI/ICF and CFF. (A and B) Significant correlation between CFF and both SICI at 2 ms and 4 ms, respectively. (C and D) Significant correlation between CFF and both ICF 10 ms and 15 ms, respectively. (SICI = short-interval intracortical inhibition, ICF = intracortical facilitation, CFF = critical flicker frequency).

4. Discussion

Our study implies three main findings. First, SICI, a measure of $GABA_A$-ergic neurotransmission, is reduced in patients with HE depending on disease severity. Second, global corticospinal excitability is reduced in patients with HE regardless of disease severity. Third, changes in both cortical GABA-ergic and glutamatergic neurotransmission are correlated with disease severity reflected by CFF.

The GABA hypothesis supposes an increase of GABA-ergic tone in HE. Our finding of reduced GABA-ergic tone in the motor cortex is in striking contrast with this hypothesis. However, an earlier study found a prolongation of cortical silent period at higher stimulation intensities which could also be mediated by reduced GABA-ergic tone and tallies with our results (Nolano et al., 1997). Consistent with our results GABA-ergic disinhibition of the motor cortex has also been described in animal studies (Cauli et al., 2009; Rangroo Thrane et al., 2013). These studies also found changes of GABA-ergic neurotransmission depending on the investigated brain region with increase of GABA-ergic tone in the cerebellum and reduction of GABA-ergic tone in the motor cortex. It is conceivable that increased cerebellar GABA-ergic inhibition leads to disinhibition of the disynaptic cerebello-thalamocortical pathway resulting in reduced GABA-ergic inhibition of the motor cortex (Cauli et al., 2009; Groiss and Ugawa, 2013). Consistent with this hypothesis we found severity dependent increase of cerebellar inhibition in patients with HE using cerebellar TMS, suggesting an increased GABA-ergic inhibition in the cerebellum of patients with HE (Hassan et al., 2019). At first sight, the results of our study seem conflicting to an earlier report by Nardone et al. who found increased GABA-ergic inhibition and reduced glutaminergic facilitation in minimal HE, which would be in line with the GABA hypothesis (Nardone et al., 2016). However, this study investigated clinical premanifest minimal HE patients, while we studied patients with manifest HE. This difference is likely the cause for the different results. Based on the assumption that GABA-ergic tone is increased in minimal HE (Nardone et al., 2016), it is possible that in earlier stages of the disease cerebellar disinhibition may be compensated by an increased GABA-ergic tone. Consistent with this notion, motor cortex disinhibition was only found in HE 2 patients and not in HE 1 patients in our study, i.e. in the more severely affected group. However, differences of the stimulation protocol regarding the number of averaged stimuli for the paired pulse TMS paradigm, which was five in the study conducted by Nardone et al. and ten in our study, also could have influenced the study results.

Although we did not find changes of glutamatergic neurotransmission in both patients with HE grade 1 and 2, we found a correlation of glutamatergic facilitation and CFF suggesting increase of cortical facilitation with progressing disease severity. This may be due to the fact that the changes were too small to be detected in a somehow arbitrary clinical grading with clear cut off values while disease severity rather represents a continuous transition. CFF, however, nicely reflects the floating changes in HE and has been shown to be an ideal marker to correlate with electophysiological measures (Timmermann et al., 2008; Butz et al., 2010, 2013; Kahlbrock et al., 2012; May et al., 2014). This correlation of glutamatergic facilitation and disease severity may be due to decreased glutamate uptake by astrocytes and increased synaptic glutamate release by hyperammonemia leading to gradual disinhibition of action potential generation and excitatory postsypaptic potentials in those more severely affected (Raabe and Gumnit, 1975; Hermenegildo et al., 2000; Häussinger and Blei, 2007; Görg et al., 2010). It is supposed that this disinhibition also leads to a disturbance of cortical functions involving postsynaptic inhibition (Raabe and Gumnit, 1975; Raabe, 1981).

Finally, despite these intracortical excitability changes revealed by paired pulse TMS we found reduced global corticospinal excitability in HE irrespective of disease stage. This is in line with earlier studies on corticospinal excitability in HE that found an increase of resting motor threshold also in patients with liver cirrhosis even without HE. It is possible that the intracortical excitability changes found in our paired pulse experiments could have been cancelled out in the recruitment curve experiments at higher stimulation intensities leading to a decreased global corticospinal excitability measured by the recruitment curve. In paired pulse TMS paradigms superficial layer 2/3 interneurons with their reciprocal connections to layer 1 neurons are supposed to play an important role especially when using lower CS intensities (Di Lazzaro and Ziemann, 2013; Di Lazzaro and Rothwell, 2014). On the other hand for the recruitment curve, where we found differences at higher intensities, excitability of layer 5 pyramidal neurons play a more important role for global corticospinal output measured with single pulse TMS (Di Lazzaro and Ziemann, 2013; Di Lazzaro and Rothwell, 2014). Astrocyte swelling and reduction of dendritic spines of corticospinal neurons in the deeper layer V of the motor cortex may be the underlying cause for decreased global corticospinal excitability (Chen et al., 2014). Although cognitive impairment is known to influence corticospinal excitability, it rather leads to hyperexcitability making it unlikely the reason for reduced corticospinal excitability which we found here (Cantone et al., 2014; Nardone et al., 2014).

It is difficult to say why serum ammonia level did not correlate with our TMS measures but correlation studies on serum ammonia level and HE severity have been rather inconsistent (Stahl, 1963; Kramer et al., 2000; Miller, 2003; Nicolao et al., 2003). Better correlation has been supposed for more severely affected HE 3 and 4 patients (Brar et al., 2016), while we investigated HE 1 and 2 patients here. For these stages CFF has been proven a reliable marker in several previous MEG studies, where CFF was correlated to general slowing of oscillatory activity (Timmermann et al., 2008; Butz et al., 2010, 2013; Kahlbrock et al., 2012; May et al., 2014). Therefore, alteration of SICI could also reflect some kind of correlate for cortical slowing.

A limitation of our study was the rather small sample size per subgroup, which could have influenced the power of our study results. Additionally, we cannot fully rule out an effect of alcohol on cortical excitability in our patients, since a relevant number of patients had alcoholic liver cirrhosis. However, a relevant effect seems unlikely since direct effects of alcohol on cortical excitability were not revealed in an earlier study. Moreover, only patients abstinent for alcohol for at least 6 weeks were included in our study (Nardone et al., 2010).

In conclusion, our data reveal a decrease of GABA-ergic neurotransmission in the primary motor cortex of HE patients challenging the classical GABA hypothesis of an increased GABA-ergic tone in HE.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

Acknowledgements

This work was supported by the German Research Foundation (SFB 974).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2019.03.011.

References

Brar R, Gupta PK, Virmani SK, Sarkar G. Correlation of serum ammonia with grades of hepatic encephalopathy. Int J Adv Med 2016;3:908–11.
Butz M, May ES, Häussinger D, Schnitzler A. The slowed brain: cortical oscillatory activity in hepatic encephalopathy. Arch Biochem Biophys 2013;536:197–203.
Butz M, Timmermann L, Braun M, Groiss SJ, Wojtecki L, Ostrowski S, et al. Motor impairment in liver cirrhosis without and with minimal hepatic encephalopathy. Acta Neurol Scand 2010;122:27–35.
Cantone M, Di Pino G, Capone F, Piombo M, Chiarello D, Cheeran B, et al. The contribution of transcranial magnetic stimulation in the diagnosis and in the management of dementia. Clin Neurophysiol 2014:1509–32.
Cauli O, Rodrigo R, Llansola M, Montoliu C, Monfort P, Piedrafita B, et al. Glutamatergic and gabaergic neurotransmission and neuronal circuits in hepatic encephalopathy. Metab Brain Dis 2009;24:69–80.
Chen J-R, Wang B-N, Tseng G-F, Wang Y-J, Huang Y-S, Wang T-J. Morphological changes of cortical pyramidal neurons in hepatic encephalopathy. BMC Neurosci 2014;15:15.
Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res 2004;154:1–10.
Chen R, Tam A, Bütefisch C, Corwell B, Ziemann U, Rothwell JC, et al. Intracortical inhibition and facilitation in different representations of the human motor cortex. J Neurophysiol 1998;80:2870–81.
Görg B, Morwinsky A, Keitel V, Qvartskhava N, Schrör K, Häussinger D. Ammonia triggers exocytotic release of L-glutamate from cultured rat astrocytes. Glia 2010;58:691–705.
Groiss SJ, Ugawa Y. Cerebellum. Handb Clin Neurol 2013;116:643–53.
Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, et al. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 2008;586:3927–47.
Hanajima R, Ugawa Y, Terao Y, Enomoto H, Shiio Y, Mochizuki H, et al. Mechanisms of intracortical I-wave facilitation elicited with paired-pulse magnetic stimulation in humans. J Physiol 2002;538:253–61.
Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, et al. Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. J Physiol 1998;509:607–18.
Hassan SS, Baumgarten TJ, Ali AM, Füllenbach N-D, Jördens MS, Häussinger D, et al. Cerebellar inhibition in hepatic encephalopathy. Clin Neurophysiol 2019. in press.
Häussinger D, Blei A. Textbook of hepatology: from basic science to clinical practice. 3rd ed. Oxford, UK: Blackwell Publishing Ltd; 2007.
Hermenegildo C, Monfort P, Felipo V. Activation ofN-methyl-D-aspartate receptors in rat brainin vivo following acute ammonia intoxication: characterization byin vivo brain microdialysis. Hepatology 2000;31:709–15.
Kahlbrock N, Butz M, May ES, Brenner M, Kircheis G, Häussinger D, et al. Lowered frequency and impaired modulation of gamma band oscillations in a bimodal attention task are associated with reduced critical flicker frequency. Neuroimage 2012;61:216–27.
Kircheis G, Hilger N, Häussinger D. Value of critical flicker frequency and psychometric hepatic encephalopathy score in diagnosis of low-grade hepatic encephalopathy. Gastroenterology 2014;146:961–9.
Kircheis G, Wettstein M, Timmermann L, Schnitzler A, Häussinger D. Critical flicker frequency for quantification of low-grade hepatic encephalopathy. Hepatology 2002;35:357–66.
Kramer L, Tribl B, Gendo A, Zauner C, Schneider B, Ferenci P, et al. Partial pressure of ammonia versus ammonia in hepatic encephalopathy. Hepatology 2000;31:30–4.
Di Lazzaro V, Rothwell JC. Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. J Physiol 2014;592:4115–28.
Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Front Neural Circ 2013;7:1–9.
May ES, Butz M, Kahlbrock N, Brenner M, Hoogenboom N, Kircheis G, et al. Hepatic encephalopathy is associated with slowed and delayed stimulus-associated somatosensory alpha activity. Clin Neurophysiol 2014;125:2427–35.
McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 2006;173:86–93.
Miller KE. Do ammonia levels correlate with hepatic encephalopathy? Am Fam Phys 2003;68:1408–10.
Nardone R, Bergmann J, Kronbichler M, Caleri F, Lochner P, Tezzon F, et al. Altered motor cortex excitability to magnetic stimulation in alcohol withdrawal syndrome. Alcohol Clin Exp Res 2010;34:628–32.
Nardone R, De Blasi P, Höller Y, Brigo F, Golaszewski S, Frey VN, et al. Intracortical inhibitory and excitatory circuits in subjects with minimal hepatic encephalopathy: a TMS study. Metab Brain Dis 2016;31:1065–70.
Nardone R, Tezzon F, Höller Y, Golaszewski S, Trinka E, Brigo F. Transcranial magnetic stimulation (TMS)/repetitive TMS in mild cognitive impairment and Alzheimer's disease. Acta Neurol Scand 2014;129:351–66.
Nicolao F, Efrati C, Masini A, Merli M, Attili AF, Riggio O. Role of determination of partial pressure of ammonia in cirrhotic patients with and without hepatic encephalopathy. J Hepatol 2003;38:441–6.

Nolano M, Guardascione MA, Amitrano L, Perretti A, Fiorillo F, Ascione A, et al. Cortico-spinal pathways and inhibitory mechanisms in hepatic encephalopathy. Electroencephalogr Clin Neurophysiol 1997;105:72–8.
Paulus W, Peterchev AV, Ridding M. Transcranial electric and magnetic stimulation: technique and paradigms. Handb Clin Neurol 2013;116:329–42.
Peurala SH, M. Müller-Dahlhaus JF, Arai N, Ziemann U. Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). Clin Neurophysiol 2008:2291–7.
Raabe W, Gumnit RJ. Disinhibition in cat motor cortex by ammonia. J Neurophysiol 1975;38:347–55.
Raabe WA. Ammonia and disinhibition in cat motor cortex by ammonium acetate, monofluoroacetate and insulin-induced hypoglycemia. Brain Res 1981;210:311–22.
Rangroo Thrane V, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M, et al. Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med 2013;19:1643–8.
Romero-Gómez M, Córdoba J, Jover R, del Olmo JA, Ramírez M, Rey R, et al. Value of the critical flicker frequency in patients with minimal hepatic encephalopathy. Hepatology 2007;45:879–85.
Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 2015;126:1071–107.
Schafer DF, Jones EA. Hepatic encephalopathy and the gamma-aminobutyric-acid neurotransmitter system. Lancet 1982;1:18–20.
Sharma P, Sharma BC, Puri V, Sarin SK. Critical flicker frequency: diagnostic tool for minimal hepatic encephalopathy. J Hepatol 2007;47:67–73.
Stahl J. Studies of the blood ammonia in liver disease. Its diagnostic, prognostic, and therapeutic significance. Ann Int Med 1963;58:1–24.
Timmermann L, Butz M, Gross J, Ploner M, Südmeyer M, Kircheis G, et al. Impaired cerebral oscillatory processing in hepatic encephalopathy. Clin Neurophysiol 2008;119:265–72.
Tokimura H, Ridding MC, Tokimura Y, Amassian VE, Rothwell JC. Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. Electroencephalogr Clin Neurophysiol 1996;101:263–72.
Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol 1992;85(6):355–64.
Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res 1996a;109:127–35.
Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996b;496:873–81.
Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W. Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. J Physiol 1998;511:181–90.