

LEARNING AND MEMORY IMPAIRMENTS IN PATIENTS WITH MINIMAL HEPATIC ENCEPHALOPATHY ARE ASSOCIATED WITH STRUCTURAL AND FUNCTIONAL CONNECTIVITY ALTERATIONS IN HIPPOCAMPUS

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Left hippocampal areas								
		Presubiculum	CA1	CA2-3	Fimbria	Subiculum	CA4-DG	Hippocampal Fissure
CVLT total learning	All participants	r=0.319 p=0.013	r=0.181 p=0.167	r=0.312 p=0.015	r=0.306 p=0.018	r=0.241 p=0.064	r=0.290 p=0.025	r= -0.066 p=0.615
	Controls	r=0.438 p=0.036	r=0.161 p=0.463	r=0.185 p=0.398	r=0.014 p=0.950	r=0.311 p=0.148	r=0.214 p=0.327	r=0.191 p=0.382
	C-NMHE patients	r=0.143 p=0.505	r=0.235 p=0.268	r=0.197 p=0.357	r=0.233 p=0.272	r=0.179 p=0.403	r=0.167 p=0.435	r=-0.085 p=0.694
	C-MHE patients	r=0.443 p=0.172	r=0.399 p=0.224	r=0.724 p=0.012	r=0.324 p=0.331	r=0.314 p=0.347	r=0.670 p=0.024	r=0.331 p=0.320
CVLT Delayed recall	All participants	r=0.438 p=0.000	r=0.313 p=0.015	r=0.375 p=0.003	r=0.257 p=0.048	r=0.360 p=0.005	r=0.372 p=0.003	r=0.053 p=0.690
	Controls	r=0.364 p=0.088	r=0.094 p=0.670	r=0.073 p=0.739	r=0.022 p=0.922	r=0.223 p=0.307	r=0.099 p=0.653	r=- 0.001 p=0.996
	C-NMHE patients	r=0.454 p=0.026	r=0.478 p=0.018	r=0.439 p=0.032	r=0.189 p=0.378	r=0.491 p=0.015	r=0.431 p=0.035	r=0.218 p=0.306
	C-MHE patients	r=0.311 p=0.352	r=0.303 p=0.365	r=0.451 p=0.164	r=0.269 p=0.423	r=0.144 p=0.673	r=0.446 p=0.170	r=0.144 p=0.672
Right hippocampal areas								
		Presubiculum	CA1	CA2-3	Fimbria	Subiculum	CA4-DG	Hippocampal Fissure
CVLT total learning	All participants	r=0.329 p=0.10	r=0.197 p=0.131	r=0.257 p=0.048	r=0.478 p=0.000	r=0.274 p=0.034	r=0.233 p=0.074	r= -0.117 p=0.374
	Controls	r=0.323 p=0.133	r=0.319 p=0.138	r=0.229 p=0.293	r=0.180 p=0.412	r=0.335 p=0.118	r=0.239 p=0.272	r=-0.011 p=0.961
	C-NMHE patients	r=0.265 p=0.211	r=0.077 p=0.722	r=0.195 p=0.361	r=0.410 p=0.047	r=0.192 p=0.370	r=0.136 p=0.526	r=-0.150 p=0.483
	C-MHE patients	r=0.437 p=0.179	r=0.609 p=0.047	r=0.566 p=0.070	r=0.345 p=0.298	r=0.367 p=0.267	r=0.474 p=0.141	r=0.097 p=0.778
CVLT Delayed recall	All participants	r=0.390 p=0.002	r=0.312 p=0.015	r=0.296 p=0.021	r=0.428 p=0.001	r=0.337 p=0.009	r=0.288 p=0.026	r=0.093 p=0.482
	Controls	r=0.129 p=0.557	r=0.286 p=0.186	r=0.087 p=0.692	r=0.089 p=0.687	r=0.213 p=0.329	r=0.100 p=0.651	r=-0.028 p=0.900
	C-NMHE patients	r=0.513 p=0.010	r=0.305 p=0.147	r=0.428 p=0.037	r=0.479 p=0.018	r=0.449 p=0.028	r=0.379 p=0.068	r=0.214 p=0.316
	C-MHE patients	r=0.324 p=0.331	r=0.430 p=0.187	r=0.274 p=0.415	r=0.356 p=0.282	r=0.218 p=0.519	r=0.218 p=0.519	r=0.046 p=0.894

Supplementary Table S1. Correlations between volumes of hippocampal regions and CVLT parameters in all the groups studied. Data show values of partial correlations, adjusting for gender. CA, Cornu Ammonis; CVLT, California Verbal Learning Test; DG: Dentate Gyrus; L, left; C-NMHE, C-MHE, cirrhotic patients without or with minimal hepatic encephalopathy, respectively; Post, posterior; R, right; ns, non-significant correlations. Significant correlations ($p < 0.05$) are in bold.

	<i>All participants</i>		<i>Controls</i>		<i>C-NMHE</i>		<i>C-MHE</i>	
<i>Hippocampal regions</i>	Ammonia	IL-18	Ammonia	IL-18	Ammonia	IL-18	Ammonia	IL-18
<i>Left hippocampal areas</i>								
L Presubiculum	ns	r=-0.27 p=0.04	ns	ns	ns	r=-0.545 p=0.006	ns	ns
L CA1	r=0.36 p=0.004	ns	ns	ns	r=0.501 p=0.013	r=-0.40 p=0.05	ns	ns
L CA2-3	ns	r=-0.298 p=0.02	ns	ns	ns	r=-0.42 p=0.04	ns	ns
L Fimbria	ns	r=-0.3 p=0.02	ns	ns	r=0.53 p=0.008	r=-0.41 p=0.04	ns	ns
L Subiculum	ns		ns	ns	ns	r=-0.45 p=0.03	ns	ns
L CA4-DG	ns	r=-0.288 p=0.02	ns	ns	r=0.415 p=0.04	r=-0.44 p=0.03	ns	ns
L Hippocampal fissure	ns	ns	ns	ns	ns	ns	ns	ns
<i>Right hippocampal areas</i>								
R Presubiculum	ns	ns	ns	ns	ns	r=-0.46 p=0.02	ns	ns
R CA1	ns	ns	ns	ns	r=0.44 p=0.03	r=-0.399 p=0.054	ns	ns
R CA2-3	ns	ns	ns	ns	ns	r=-0.42 p=0.04	ns	ns
R Fimbria	ns	r=-0.32 p=0.012	ns	ns	r=0.47 p=0.02	r=-0.37 p=0.07	ns	ns
R Subiculum	ns	ns	ns	ns	ns	r=-0.5 p=0.013	ns	ns
R CA4-DG	ns	ns	ns	ns	ns	r=-0.48 p=0.02	ns	ns
R Hippocampal fissure	ns	ns	ns	ns	ns	ns	ns	ns

Supplementary Table S2. Significant correlations between volumes of hippocampal regions and biochemical markers in all the groups studied. Data show values of partial correlations adjusting for gender. CA, Cornu Ammonis; CVLT, California Verbal Learning Test; DG: Dentate Gyrus; L, left; C-NMHE, C-MHE, cirrhotic patients without or with minimal hepatic encephalopathy, respectively; Post, posterior; R, right; ns, non-significant correlations. Significant correlations ($p < 0.05$) are in bold.

	Controls (n=18)	C-NMHE patients (n=19)	C-MHE patients (n=10)	C-NMHE <i>P</i> vs. controls	C-MHE <i>P</i> vs. controls	C-MHE <i>P</i> vs. C-NMHE
Age (range)	60 ± 1 (50-73)	63 ± 2 (50-81)	65 ± 4 (49-85)	ns	ns	ns
Gender (Male/Female)	10 / 8	14 / 5	9 / 1			
Child Pugh A/B/C	-	17 / 2 / 0	7 / 3 / 0			
Alcohol / HCV/ others	-	7 / 10/ 2	4 / 3 / 3			
PHES score	1 ± 0.2	-1.1 ± 0.3	-7.5 ± 1.1	0.005	<0.001	<0.001
CVLT^a						
<i>Learning trial 1</i>	6.2 ± 0.3	3.9 ± 0.5	3.9 ± 0.3	<0.001	0.004	1.000
<i>Learning trial 2</i>	8.9 ± 0.4	6.7 ± 0.7	5.8 ± 0.3	0.019	0.005	1.000
<i>Learning trial 3</i>	10.8 ± 0.5	7.8 ± 0.6	5.6 ± 0.6	0.002	<0.001	0.07
<i>Learning trial 4</i>	11.7 ± 0.5	9.1 ± 0.6	6.1 ± 0.6	0.007	<0.001	0.008
<i>Learning trial 5</i>	12.3 ± 0.5	10.3 ± 0.8	5.8 ± 0.8	0.12	<0.001	0.001
<i>Total Learning</i>	50 ± 1	38 ± 3	27 ± 2	0.001	<0.001	0.017
<i>Delayed Recall</i>	11.4 ± 0.6	9.2 ± 0.9	6.1 ± 0.4	0.15	0.001	0.06
<i>Recognition</i>	14.7 ± 0.4	14.9 ± 0.3	12.9 ± 0.6	1.000	0.026	0.009
Biochemical determinations						
Blood ammonia (μM)	9.5 ± 0.8	20 ± 5	32 ± 7	0.285	0.011	1.000
Plasma cGMP (pmol/ml)	4.2 ± 0.2	8 ± 1.2	13 ± 1.4	0.015	<0.001	0.008
Serum IL-6 (pg/ml)	1.5 ± 0.1	2.6 ± 0.2	4.1 ± 0.6	0.005	<0.001	0.001
Serum IL-18 (pg/ml)	145 ± 19	273 ± 23	321 ± 26	<0.001	<0.001	0.570

Supplementary Table S3. Main demographic, clinical and neuropsychological characteristics of participants in whom functional connectivity was analyzed.

Values are expressed as mean ± SEM. C-NMHE and C-MHE, cirrhotic patients without and with Minimal Hepatic Encephalopathy, respectively; PHES, Psychometric Hepatic Encephalopathy Score; ^aCVLT, Spanish adapted version of California Verbal Learning Test. Child Pugh Score is derived from a score of 1–3 given for severity of ascites, hepatic encephalopathy, INR, albumin, and bilirubin. The higher the score, the more severe the liver disease. Differences between groups were analyzed using one-way ANOVA followed by a post-hoc multiple comparisons Bonferroni test. Using Bonferroni correction for multiple comparisons (n=3), *P* values <0.016 were considered significant (in bold).

Left hippocampal areas								
		Presubiculum	CA1	CA2-3	Fimbria	Subiculum	CA4-DG	Hippocampal Fissure
	All participants	ns	ns	ns	ns	ns	ns	ns
CVLT total learning	Controls	ns	ns	ns	ns	ns	ns	ns
	C-NMHE patients	ns	ns	ns	ns	ns	ns	ns
	C-MHE patients	ns	ns	ns	ns	ns	ns	ns
	All participants	r=0.38 p=0.009	r=0.315 p=0.03	r=0.365 p=0.015	ns	r=0.338 p=0.02	r=0.363 p=0.013	ns
CVLT Delayed recall	Controls	ns	ns	ns	ns	ns	ns	ns
	C-NMHE patients	r=0.563 p=0.015	r=0.499 p=0.035	r=0.464 p=0.052	ns	r=0.585 p=0.01	r=0.473 p=0.048	ns
	C-MHE patients	ns	ns	ns	ns	ns	ns	ns
Right hippocampal areas								
		Presubiculum	CA1	CA2-3	Fimbria	Subiculum	CA4-DG	Hippocampal Fissure
	All participants	ns	ns	ns	ns	ns	ns	ns
CVLT total learning	Controls	ns	ns	ns	ns	ns	ns	ns
	C-NMHE patients	ns	ns	ns	ns	ns	ns	ns
	C-MHE patients	ns	ns	ns	ns	ns	ns	ns
	All participants	r=0.350 p=0.02	ns	r=0.31 p=0.04	r=0.322 p=0.03	r=0.282 p=0.058	r=0.316 p=0.03	ns
CVLT Delayed recall	Controls	ns	ns	ns	ns	ns	ns	ns
	C-NMHE patients	r=0.639 p=0.004	ns	r=0.481 p=0.04	r=0.536 p=0.02	r=0.523 p=0.03	ns	ns
	C-MHE patients	ns	ns	ns	ns	ns	ns	ns

Supplementary Table S4. Correlations between volumes of hippocampal regions and CVLT parameters in participants in whom functional connectivity was analyzed (18 controls, 19 C-NMHE and 10 C-MHE). Data show values of partial correlations adjusting for gender. CA, Cornu Ammonis; CVLT, California Verbal Learning Test; DG: Dentate Gyrus; L, left; NMHE, patients without minimal hepatic encephalopathy; Post, posterior; R, right. Significant correlations ($p < 0.05$) are in bold.

		<i>FC differences C-MHE < C-NMHE</i>		<i>FC differences C-MHE < HC</i>
		L Presubiculum-L Precuneus	L Subiculum- bilateral PC	L Presubiculum- bilateral PC
PHES	All participants	r=0.438 p=0.002	r=0.415 p=0.004	r=0.458 p=0.001
	Controls	ns	ns	ns
	C-NMHE patients	ns	ns	ns
	C-MHE patients	ns	ns	ns
CVLT-Total learning	All participants	r=0.210 p=0.161	r=0.517 p<0.001	r=0.347 p=0.018
	Controls	ns	ns	ns
	C-NMHE patients	ns	r=0.523 p=0.026	ns
	C-MHE patients	ns	ns	ns
CVLT-Delayed recall	All participants	r=0.254 p=0.088	r=0.411 p=0.004	r=0.248 p=0.096
	Controls	ns	ns	ns
	C-NMHE patients	ns	r=0.475 p=0.046	ns
	C-MHE patients	ns	r=0.564 p=0.113	ns
CVLT- Recognition	All participants	ns	ns	ns
	Controls	ns	ns	ns
	C-NMHE patients	ns	ns	ns
	C-MHE patients	ns	r=0.657 p=0.05	ns

Supplementary Table S5. Significant correlations between functional connectivity of hippocampal seeds and neuropsychological measures in patients. Data show values of partial correlations adjusting for gender. CVLT, California Verbal Learning Test; FC, functional connectivity; HC, healthy controls; L, left; C-NMHE, patients without minimal hepatic encephalopathy; C-MHE, patients with minimal hepatic encephalopathy; PC, precuneus; ns, non-significant correlations. Significant correlations (p<0.05) are in bold.