SUPPLEMENTARY MATERIAL

Patients with Minimal Hepatic Encephalopathy Show Altered Thermal Sensitivity and Autonomic Function

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Contents:

Supplementary methods

Quantitative Sensory Testing (QST)

The calculation of the sensory thresholds was done by administering a series of non-invasive vibratory or thermal stimuli, corresponding to a set of 25 standardized vibratory and thermal stimulation levels, according to a one-time-period 4, 2, 1 Stepping Algorithm (Fig. S2) (*Dyck P, Zimmerman I, Gillen D, Johnson D, Karnes J, O'Brien P. Cool, warm, and heat-pain detection thresholds: Testing methods and inferences about anatomic distribution of receptors. Neurology. 1993; 43: 1500-1508*). This algorithm determines how the stimuli are presented. During a given test, subsequent stimuli may be dependent on a patient's response. These tests have a total of 20 stimulus trials; each trial corresponds to one-time period. During this time period, the stimulus may or may not be delivered (five periods of null stimuli are placed randomly to prevent false results). The green light will flash on the Patient Cue Device, signalling the beginning of a trial. Then, a "1" will be presented, signalling the time period. The subject must try to determine whether a stimulus (vibration or thermal) was delivered. The patient then answers by pressing "yes" or "no" on the Patient Response Device for cooling and vibration tests. For heat-pain testing the subject answers a number from 0 to 10, 0 being no pain and 10 being maximum pain possible.

The VDT stimuli are administered by the Vibration Stimulator (Fig. S1A) consisting of a galvanometer, set at 125 cycles per second, variable between 0 and 350 micrometres. The CDT and HPDT tests are done by the thermal Stimulator (Fig. S1B), a ceramic plate, held in place by a Velcro strap, which produces a specified temperature, which can be varied from 8.0 to 50.0 degrees C, with accuracy of 1.25 to 0.25 degrees C, on a 9.0-square-centimeter stimulating surface (traceable to National Institute of Standards and Technology, NIST, standards). Prior to testing, the Thermal Stimulator adjusts itself to match the patient's baseline skin temperature. For statistical normalization purposes, the baseline temperature was 30 ºC (for Cooling test) or 34 ºC (for Heat-Pain test). For high-magnitude thermal (warming) stimuli, a holding time is added to the waveform so that the absolute temperature is typically limited to 50ºC. The plateau lengthens the time that the stimulus is administered, providing more heat over time, ensuring the same physiologic sensation as a higher pyramidal-shaped waveform. For high-magnitude thermal (cooling) stimuli, the temperature is limited to 8ºC.

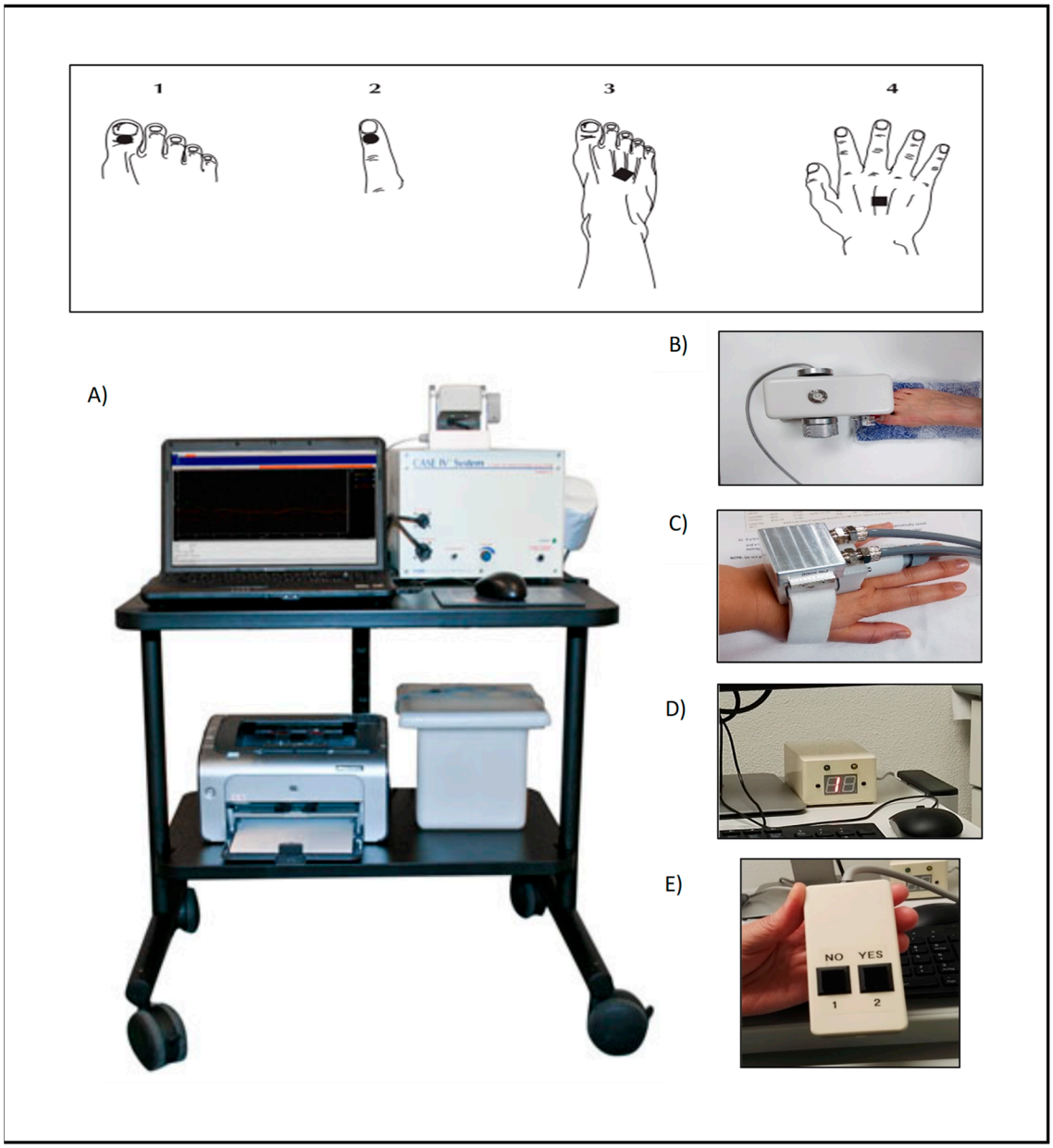

Figure S1. Quantitative Sensory Test components. The CASE IV system (**A**) consists of: The Vibration Stimulator (**B**), a galvanometer, set at 125 cycles per second, variable between 0 and 350 micrometres. The thermal Stimulator (**C**), a ceramic plate, held in place by a Velcro strap, which produces a specified temperature, which can be varied from 8.0 to 50.0 degrees C. These stimulus apparatuses are placed either on the hand or foot, 1 and 2 being for vibration detection tests and 3 and 4 being for thermal tests. The subject is then asked to pay attention to the box (**D**), when the light turns on they must be prepared to attend to the stimulus which is administered or not when the one appears. When the number turns off, the subject must respond if a stimulus was administered yes or no on the remote device (**E**).

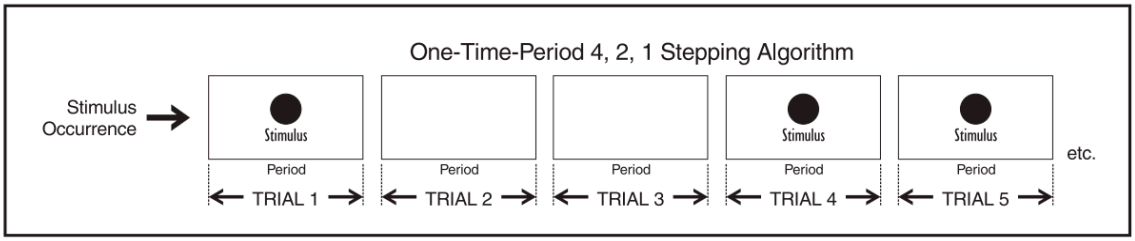

Figure S2. One – Time – Period 4, 2, 1 Stepping Algorithm. This algorithm determines how the stimuli are presented. During a given test, subsequent stimuli may be dependent on a patient's response. These tests have a total of 20 stimulus trials; each trial corresponds to one time period. During this time period, the stimulus may or may not be delivered (five periods of null stimuli are placed randomly to prevent false results) (modified form *Dyck P, et al. Cool, warm, and heat-pain detection thresholds: Testing methods and inferences about anatomic distribution of receptors. Neurology. 1993; 43:1500-1508*).

Neurophysiological studies of large fibers: nerve conduction study

Protocol: It is advisable that each laboratory should elaborate their own independent protocol of neurophysiological evaluation due to the differences which exist between testing machines, techniques, and individual characteristics of the study population.

The conduction study protocol used for the diagnosis of polyneuropathy, in this case, was based on those described by Stålberg [28] and Preston [29].

The studied sensory nerves were the:

- Unilateral ulnar.
- Unilateral superficial radial.
- Bilateral sural.
- Bilateral superficial peroneal nerves.

The motor nerves explored were the:

- Unilateral ulnar.
- Bilateral peroneal nerves.
- Bilateral posterior tibial nerves.

In addition, F waves of the unilateral cubital nerve and bilateral posterior tibial nerve were assessed. Also, autonomic testing was undertaken by assessing the R-R interval and cutaneous sympathetic response (CSR).

Reference values were also based on those described by Preston [29] and then adapted to our own normal values obtained from our laboratory and study population. These protocols [28] define that an axonal sensory polyneuropathy presents a series of characteristics: An amplitude reduction of sensory nerve conduction, with a greater affection of distal nerves in lower extremities (in more severe polyneuropathies motor nerve conduction amplitude may also be affected). Nerve conduction velocity is normal or can be slightly slower. F waves can have a delayed or diminished persistence. And autonomic tests are frequently altered.

Parameters: The parameters used to study evoked responses of nerve conduction were:

- Initial latency (measured nerve conduction time, in milliseconds, from which the stimuli begins, to the initial moment of the evoked response).
- Amplitude (median value, in millivolts, of the negative peak and positive peak of the evoked response, it informs us on the number of stimulated axons).
- Conduction velocity (expressed in m/s, calculated by measuring two stimulated points of the same nerve and dividing it by the difference between proximal latency and distal latency).

When studying nerve conduction parameters (sensory and motor) it was considered indicative of alteration when amplitude was diminished, conduction velocity was diminished, or latency was increased, according to established reference values *(Iriarte, F.; Artieda, J. Manual of Clinical Neurophysiology. Panamericana.* ***2012****, pp. 118-123).*

Table S1. QST and autonomic testing parameters comparing males and females in the control group

Values are expressed as mean ± SD. Differences between groups were analyzed by Student's T-test. Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just noticeable differences.

QST parameters	**Test site**	**Males**	**Females**	***P*-values**
Vibration detection (JND)	hand	6.0±0.9	7.0±0.5	0.332
	foot	13.0±1.0	13.2±0.9	0.887
Cooling detection (JND)	hand	6.7±0.5	6.8±0.4	0.864
	foot	7.9±0.4	8.3±0.6	0.660
Heat pain 0.5 (JND)	hand	15.5±1.0	16.6±0.8	0.377
	foot	17.2±0.8	18.2±0.5	0.252
Heat pain 5.0 (JND)	hand	20.2±0.9	19.9±0.7	0.792
	foot	21.5±0.6	21.2±0.5	0.748
Vibration detection time (s)	hand	128.56±2.9	127.16±1.5	0.637
	foot	126.5±2.6	128.0±2.6	0.712
Cooling detection time (s)	hand	143.9±2.0	138.8±1.6	0.060
	foot	144.0±3.1	143.6±3.3	0.942
Heat pain time (s)	hand	113.9±18.2	110.0±10.4	0.843
	foot	130.5±15.3	121.5±12.2	0.658
Autonomic testing				
R-R Interval variation (%)	Basal	4.7±2.72	2.8±0.6	0.559
	Hyperventilation	18.2±4.9	9.3±1.7	0.163
	Valsalva	14.6±7.9	11.9±1.7	0.759
	Orthostatic test	14.5±8.1	6.2±2.21	0.176
Cutaneous sympathetic response	Amplitude	5.1±1.7	3.8±0.5	0.351
	Latency	1.33±0.02	1.42±0.05	0.362

Table S2. Parameters of sensory and motor nerve conduction in controls and patients with normal sural nerve.

Values are expressed as mean ± SEM. MHE, NMHE, patients with and without minimal hepatic encephalopathy, respectively. Differences between groups were analyzed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test. Differences compared to control group are indicated by asterisks: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.

Parameters	Control	NMHE patients *P* vs. Control	MHE patients *P* vs. Control	MHE patients *P* vs. NMHE	ANOVA Global *P*-values
Sensory nerve conduction					
Ulnar sensory nerve amplitude (μV)	13.1±1.4	9.8±0.6	10.0±1.0	ns	0.042
Radial sensory nerve amplitude (μV)	31.5±2.0	24.0±1.8*	33.2±2.2	0.010	0.004
Sural sensory nerve amplitude (μV)	26.5±2.1	24.5±1.7	20.6±0.9*	ns	0.059
Superior peroneal amplitude (μV)	18.2±1.3	13.3±1.3	15.5±0.7*	ns	0.020
Ulnar sensory nerve latency (ms)	1.68±0.03	1.88±0.04**	1.93±0.05**	ns	0.001
Radial sensory nerve latency (ms)	1.28±0.03	1.43±0.05	1.50±0.05*	ns	0.009
Sural sensory nerve latency (ms)	1.74±0.05	1.81±0.06	1.94±0.05	ns	0.092
Superior peroneal latency (ms)	1.92±0.06	2.05±0.08	2.14±0.08	ns	0.202
Ulnar sensory nerve conduction velocity (m/s)	58.1±0.8	56.3±0.8	54.6±0.7*	ns	0.029
Radial sensory nerve conduction velocity (m/s)	66.0±1.1	62.4±1.0	61.9±1.3	ns	0.028
Sural sensory nerve conduction velocity (m/s)	58.9±1.1	58.2±0.9	56.5±1.1	ns	0.342
Superior peroneal conduction velocity (m/s)	56.5±0.8	56.4±0.9	55.9±1.0	ns	0.921
Motor nerve conduction					
Ulnar motor nerve amplitude (μV)	14.6±0.7	14.3±0.5	14.9±0.7	ns	0.799
Peroneal motor nerve amplitude (μV)	7.4±0.5	7.9±0.4	7.0±0.5	ns	0.475
Tibial motor nerve amplitude (μV)	17.4±1.1	15.6±1.0	18.8±1.9	ns	0.239
Ulnar motor nerve latency (ms)	2.18±0.06	2.52±0.07**	2.69±0.17*	ns	0.002
Peroneal motor nerve latency (ms)	3.29±0.11	3.56±0.09	3.52±0.16	ns	0.185
Tibial motor nerve latency (ms)	3.39±0.12	3.43±0.10	3.42±0.10	ns	0.953
Ulnar motor nerve conduction velocity (m/s)	61.3±1.0	56.2±1.0**	53.1±0.4****	0.018	<0.001
Peroneal motor nerve conduction velocity (m/s)	47.4±1.1	43.9±0.7*	43.0±0.8**	ns	0.004

Table S3. Comparison of QST parameters and autonomic testing between patients with alcoholic etiology and with other etiologies, in the group of patients with normal sural nerve.

		Etiology		
QST parameters	**Test site**	**alcohol**	**other**	***P*-values**
Vibration detection (JND)	hand	9 ± 4	9.7 ± 2	0.62
	foot	16.2 ± 2.3	16.5 ± 2.4	0.72
Cooling detection (JND)	hand	9.5 ± 3	10.5 ± 3	0.35
	foot	12.4 ± 5	13.2 ± 4	0.61
Heat pain 0.5 (JND)	hand	19.4 ± 3.5	17.4 ± 3	0.10
	foot	19.8 ± 1.5	19.7 ± 2	0.92
Heat pain 5.0 (JND)	hand	22 ± 2.4	22 ± 2.2	0.55
	foot	22 ± 1.2	22 ± 1	0.81
Vibration detection time (s)	hand	132 ± 10	134 ± 12	0.67
	foot	133 ± 7	136 ± 11	0.44
Cooling detection time (s)	hand	157 ± 34	148 ± 14	0.34
	foot	183 ± 80	170 ± 30	0.56
Heat pain time (s)	hand	163 ± 55	167 ± 77	0.87
	foot	165 ± 63	152 ± 46	0.55
Autonomic testing				
R-R Interval variation (%)	Basal	3.6 ± 3.8	3.3 ± 2.7	0.80
	Hyperventilation	5.1 ± 3.7	8.2 ± 7	0.17
	Valsalva	11.4 ± 9.3	10.6 ± 7	0.79
	Orthostatic test	4.9 ± 5.2	5 ± 4.9	0.96
Cutaneous sympathetic response	Amplitude	1.9 ± 1.5	2.8 ± 1.6	0.13
	Latency	1.5 ± 0.2	1.5 ± 0.2	0.48

Values are expressed as mean ± SD. Differences between groups were analyzed by Student's T-test. QST, quantitative sensory test; s, seconds, JND, Just noticeable differences.

Table S4. Contribution of liver disease severity to results observed in patients with normal sural nerve

		Child-Pugh		
QST parameters	**Test site**	**A**	**B**	***P*-values**
Vibration detection (JND)	hand	9.5 ± 2.8	8.9 ± 2.3	0.67
	foot	16.2 ± 2.3	17.1 ± 2.1	0.46
Cooling detection (JND)	hand	9.9 ± 3	10.5 ± 4	0.67
	foot	13.3 ± 4	10.8 ± 2	0.21
Heat pain 0.5 (JND)	hand	18 ± 3.5	19 ± 1.5	0.27
	foot	19.7 ± 2	19.8 ± 0.5	0.93
Heat pain 5.0 (JND)	hand	22.4 ± 2.4	21.6 ± 1	0.49
	foot	22 ± 1	22 ± 1.2	0.96
Vibration detection time (s)	hand	134 ± 11	130 ± 10	0.50
	foot	135 ± 10	133 ± 7	0.73
Cooling detection time (s)	hand	153 ± 26	151 ± 13	0.87
	foot	179 ± 61	157 ± 14	0.42
Heat pain time (s)	hand	168 ± 72	154 ± 45	0.69
	foot	154 ± 50	176 ± 77	0.46
Autonomic testing				
R-R Interval variation (%)	Basal	3.2 ± 3	4.6 ± 5	0.47
	Hyperventilation	6 ± 4	11 ± 10	0.33
	Valsalva	11 ± 8	13 ± 9	0.56
	Orthostatic test	5 ± 5	4 ± 2	0.49
Cutaneous sympathetic response	Amplitude	2.6 ± 1.5	1.8 ± 2	0.36
	Latency	1.4 ± 0.2	1.7 ± 0.2	0.06

Values are expressed as mean ± SD. Differences between groups were analyzed by Student's T-test.
Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just noticeable differences.

Table S5. Comparison of QST parameters and autonomic testing in patients with normal sural nerve amplitude with and without diabetes.

QST parameters	Test site	Without Diabetes	With Diabetes	*P*-values
Vibration detection (JND)	hand	9.6 ± 3	9 ± 3	0.63
	foot	16 ± 2	15 ± 3	0.25
Cooling detection (JND)	hand	10 ± 3	10 ± 3	0.98
	foot	12.5 ± 4	13.7 ± 4	0.51
Heat pain 0.5 (JND)	hand	17.5 ± 3.4	20 ± 3	0.09
	foot	19.7 ± 2	19.2 ± 1	0.64
Heat pain 5.0 (JND)	hand	22 ± 2	23 ± 3	0.15
	foot	21.8 ± 1	22 ± 1	0.63
Vibration detection time (s)	hand	134 ± 11	131 ± 12	0.55
	foot	135 ± 10	133 ± 10	0.68
Cooling detection time (s)	hand	151 ± 26	153 ± 17	0.89
	foot	176 ± 59	171 ± 41	0.83
Heat pain time (s)	hand	162 ± 63	180 ± 78	0.55
	foot	163 ± 56	140 ± 38	0.33
Autonomic testing				
R-R Interval variation (%)	Basal	3.3 ± 2.7	3.6 ± 4.5	0.85
	Hyperventilation	7 ± 6	5 ± 4	0.44
	Valsalva	10.6 ± 7	11 ± 10	0.90
	Orthostatic test	5.7 ± 5.3	2.4 ± 2	0.12
Cutaneous sympathetic response	Amplitude	2.9 ± 2	1.6 ± 1	0.10
	Latency	1.5 ± 0.2	1.5 ± 0.2	0.91

Values are expressed as mean ± SD. Differences between groups were analyzed by Student's T-test.
Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just noticeable differences.