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Laser evoked potentials in fibromyalgia with peripheral small fiber involvement

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HIGHLIGHTS

- Multichannel laser evoked potentials analysis is not coherent with small fibers reduction in Fibromyalgia patients.
- There was no correlation between the degree of denervation and the strength of late wave dipole activation.
- A correlation was noted between anxiety, depression, fibromyalgia invalidity, pain diffusion and P2 wave sources.

ABSTRACT

Objective: To evaluate multichannel laser evoked potentials (LEPs) in patients with fibromyalgia (FM) and small fiber impairment.
Methods: We recorded LEPs using 65 electrodes in 22 patients with FM and proximal denervation, 18 with normal skin biopsy, and 7 with proximal and distal intraepidermal nerve fiber density (IENFD) reduction. We considered the amplitude and topographical distribution of N1, N2 and P2 components, and habituation of N2 and P2 waves. The sLORETA dipolar analysis was also applied. We evaluated 15 healthy subjects as controls.
Results: We observed reduced amplitude of the P2 component in FM group, without a topographic correspondence with the prevalent site of denervation. Decreased habituation of P2 prevailed in patients with reduced IENFD. The cingulate cortex and prefrontal cortex, were activated in the FM group, without correlation between the degree of denervation and the strength of late wave dipoles. A correlation was noted between anxiety, depression, fibromyalgia invalidity, and pain diffusion.
Conclusions: The amplitude and topography of LEPs were not coherent with epidermal nerve fiber density loss. They supposedly reflected the clinical expression of pain and psychopathological factors.
Significance: Multichannel LEPs are not the expression of small fiber impairment in FM. Rather, they reflect the complexity of the disease.

1. Introduction

In recent years, novel data on central and peripheral nervous system (PNS) involvement have progressively changed the general view of fibromyalgia (FM) pathophysiology.

Researchers have identified a dysfunction in nociceptive input processing at the central level as the primary cause of FM, sustained by several clinical, psychophysiological, and neurophysiological evidences (Yunus, 2015).

Other data from different laboratories have contributed to the peripheral origin of FM. Interestingly, small fiber neuropathy (SFN) has been observed in several patients who underwent skin biopsy. A recent meta-analysis reported on a 49% prevalence of SFN across studies on FM populations (Grayston et al., 2019).

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Several studies have employed pain-related evoked potentials in FM to assess the functional status of nociceptive pathways. Laser-evoked potentials (LEPs) explores specifically nociceptive afferent activation. In the first LEPs studies in FM patients, they were found increased in amplitude (Gibson et al., 1994). Reduced habituation during repetitive sessions of stimulation was also observed (de Tommaso et al., 2011). Similar patterns have been reported in diseases frequently associated with FM, such as migraine, characterized by a dysfunction of pain processing at the central level (de Tommaso and Vecchio, 2013). Reduced habituation of vertex LEPs characterizes FM patients, including those with small fiber involvement, despite varying amplitude (de Tommaso et al., 2014b).

Cortical potentials obtained with a special electrode designed to preferentially activate A-delta fibers (pain-related evoked potentials) demonstrated reduced amplitude in a small cohort of patients with FM, who also presented with proximal small fiber denervation and neuropathic features at the quantitative sensory testing (QST) (Üçeyler et al., 2013). This study will contribute to the understanding of the relevance of small fiber pathology in FM towards a neuropathic origin of pain. A recent German study confirmed a congruent association between peripheral denervation, as assessed by skin biopsy and corneal confocal microscopy, PREP reduced amplitude and severe FM picture, suggesting that small fiber pathology could have a clinical impact in a subgroup of patients (Evdokimov et al., 2019).

Moreover, in most studies conducted in FM with LEPs, the LEP pattern was incongruent with the peripheral involvement of A-delta fibers. Furthermore, the LEP vertex complex amplitude was within and even above normal limits in most patients (Van Assche et al., 2020). The presence of small fiber denervation did not influence pain sensitivity and cortical responses, as assessed by QST and laser-evoked potentials in a cohort of patients with FM (Fasolino et al., 2020). It was hypothesized that reduced habituation could be a central mechanism capable of compensating for peripheral afferent loss and in contrast the LEP amplitude reduction because of weak peripheral input (Vecchio et al., 2020).

Most of the above-mentioned studies employed few electrodes, and did not assess the topographical distribution and dipolar sources of pain-related evoked potentials in FM, considering peripheral denervation.

In painful neuropathies, LEP amplitudes correlate with the degree of skin afferent loss (Casanova-Molla et al., 2011). It is unclear if the amplitude of pain-related responses, detected with multichannel LEPs, could reveal such correlation in patients with FM. Accordingly, the strength of the dipolar sources could be congruent with the degree of denervation. Alternatively, other factors, such as psychopathological factors, could modulate cortical activation under painful stimulation.

To confirm or refute the congruence between peripheral small afferent loss and LEP amplitude and cortical sources in FM, we evaluated multichannel LEPs in sub-cohorts of patients with different degree of small fibers involvement, reported in a recent evaluation (Vecchio et al., 2020). Multichannel topographic and cortical source LEP analysis could give an aid in understanding the coherence of LEPs features with skin biopsy results in FM. For these purposes, we used MATLAB Letswave and standardized low-resolution brain electromagnetic tomography (sLORETA) multidipole model software.

2. Methods

2.1. Subjects

We evaluated multichannel LEPs in a subgroup of patients with FM, recently reported in an observational study (Vecchio et al., 2020). That study included data on 81 patients consecutively selected for skin biopsy and LEP study from January to December 2017. In the last six months of recruitment, we used multichannel LEPs. For the present analysis, we included 47 patients diagnosed with FM, according to the American College of Rheumatology (ACR) criteria (Wolfe et al., 1990, 2010). For LEP analysis, we considered 15 healthy age-and sex-matched controls (Table 1).

The inclusion criteria were as follows: (i) diagnosed with FM according to the 1990 American College Rheumatology-ACR- criteria, revised in 2010 (Wolfe et al., 2010, 1990) and (ii) aged between 18 years and 75 years. The exclusion criteria were as follows: (i) education below 8 years and (ii) any cause of PNS or central nervous system (CNS) diseases, including spinal cord diseases and radiculopathies, diabetes, active thyroid insufficiency, renal failure, active autoimmune diseases and/or inflammatory arthritis, systemic connective tissue disease, psychiatric conditions, according to DSMV, a history of cancer, and current use of drugs acting on the CNS or chronic opioid therapy. We advised the patients to begin treatment with antiepileptic drugs or antidepressants following their clinical, neurophysiological, and skin biopsy assessments. Patients under analgesics were instructed to avoid analgesic use 24 h prior to LEP recording to avoid any effect on the amplitudes (Truini et al., 2010). All selected subjects provided their informed consent. The study was approved by the Ethics Committee of the Bari Policlinico General Hospital.

2.2. Clinical assessment

All patients underwent focused interviews, including thorough bedside sensory testing and standard neurological examinations (Vecchio et al., 2020). We considered the fibromyalgia-linked invalidity questionnaire (Bidari et al., 2014) (FIQ), the Wide Pain Index (WPI) included in the recent ACR diagnostic criteria (Wolfe et al., 1990), the Zung Self-Rating Depression Scale (SDS) (Zung, 1980), and Anxiety Scale (SAS) (Zung, 1976).

2.3. Nerve conduction study

We performed the nerve conduction study (NCS) according to standard methods of recording and superficial electrodes (Kimura, 2013), using a MICROMED Myoquick 2 channel device (Micromed, Mogliano Veneto, Italy). We measured the right antidromic sensory sural nerve, motor posterior tibial and peroneal nerve conduction velocity, and action potential amplitudes (Devigili et al., 2008), and compared them with normative reference values from our laboratory.

2.4. Skin biopsy

The methods followed have been previously reported (Vecchio et al., 2020). All patients underwent 3-mm punch biopsies from the thigh and distal leg following an intradermal injection of 1% xylocaine. Briefly, the specimens were fixed in 2% paraformaldehyde–lysine–sodium periodate, at 4 °C overnight, following which they were cryoprotected, serially cut with a cryostat, and immunostained using polyclonal anti-protein gene product 9.5 (Ultraclone Ltd). We calculated the intraepidermal nerve fiber density on three non-consecutive central sections by bright-field microscopy, using a stereology workstation (Olympus BX50, PlanApo oil-objective 40x/NA = 1.0), and compared them to sex-and age-adjusted normative values (Devigili et al., 2008; Lauria et al., 2010). On the basis of a consistent difference with normative data (Lauria et al, 2010) for more than 2 SD, patients were classified into those with proximal reduced IENFD-FMP-, distal and proximal reduced IENFD-FMD- and normal IENFD-FMN-.

Table 1
Demographic and clinical data of FM patients with normal skin biopsy (FMN), proximal denervation (FMP) and proximal and distal denervation (FMP). In brackets results of Bonferroni test are reported.

		AGE (years)	WPI	DURATION (years)	IENFD – P (fibers/mm)	IENFD – D (fibers/mm)
FMN 15f 3 m	M	43.56 (19–65)	11.93	10.27	14.02	10.67
	SD	11.41	4.86	7.17	3.98	2.98
FMP 18f 4 m	M	50.82 (20–69)	11.61	10.23	8.24	8.02
	SD	6.65	5.53	6.83	2.6	2.49
FMD 7f	M	45.71 (21–67)	12.83	10.42	8.9	6.51
	SD	10.88	5.6	8.67	3.79	3.33
C 10f 5 m	M	44.33 (19–66)				
	SD	14.29				
ANOVA	F	1.84	0.110	0.002	19.81	7.14
	DF	3	2	2	2	2
	p	n.s	n.s.	n.s	<0.001 Bonferroni: FMNvsFMP-FMD < 0.001	0.002 Bonferroni FMDvsFMN-FMP < 0.01

NRS: Numerical Rating Scale 0–10.
WPI: Wide Pain Index.
Proximal Intra Epidermal Nerves Fibers Density IENFD-P.
Distal Intra Epidermal Nerves Fibers Density IENFD – D.

2.5. Stimulation procedure

The details of the stimulation procedure have been reported earlier (Vecchio et al., 2020). Briefly, the pain stimulus consisted of pulses (wavelength 10.6 lm) generated by a CO2 laser (Neurolas Electronic Engineering, Florence, Italy). A series of 30 consecutive laser stimuli was then delivered to any stimulation site at an intensity level set at one step (1.5 W) above the pain threshold, at an inter-stimulus interval of 10 s. To avoid damage to the skin, fatigue, or sensitisation of nociceptors, we shifted the irradiated spot after each stimulus. An interval of 5 min separated the single stimulation series. We stimulated the right hand-back, distal thigh at 20 cm below the iliac spine, and the dorsum of the foot in a random order. A proximal and distal leg site was chosen according to the site of the biopsy. The hand was also stimulated. However, in the present study, we reported on the topographical analysis of LEPs obtained by foot and thigh stimulation, in accordance with the sites of skin biopsy. The patients were requested to pay attention to the stimulus intensity, and the color scale from 0 (white color-no pain) to 100 (intense red-the most intense pain) was preventively presented. Following a single stimulation series, we requested the patients to rate the perceived pain using the 0–100 Visual Analog Scale (VAS). To exclude the involvement of large myelinated fibers, all patients underwent nerve conduction studies (Vecchio et al., 2020).

2.6. Recording procedure

We used a montage with 65 scalp electrodes, referred to as the nasion. Two additional electrodes were positioned above the eyebrows for electrooculogram (EOG) recording. Moreover, the ground electrode was located at the frontopolar zone.

2.7. LEP analysis

We conducted preprocessing in MATLAB using the EEGLAB 14_1_1 tool. The data were initially high-pass filtered at 1 Hz to remove the slow drifts. Subsequently, we applied a notch filter at 50 Hz (L: 48, H: 52) to remove the power line noise artifacts. To pre-compute the channel measures, we deleted the EOG-related artifact components of the independent component analysis and performed a spherical interpolation of the missing channels. Bad channels were identified using a semiautomatic method, based on visual detection and channel statistics. Channels presenting the distributions of potential values farther from the Gaussian distribution were removed. We pre-computed the LEPs in the time interval of 800 ms post stimuli, using a 70 Hz low-pass filter, removing the baseline and considering the 100 ms preceding the laser stimulus. For the patients and controls, we averaged 30 trials for each stimulation site. We used the Letswave tool version.7. Following the single track visual analysis, we identified major LEP waves at predetermined time intervals (N1 Hand 160–200 ms; N2 Hand 210–250 ms; P2 Hand 310–360 ms N1 foot 178–204 ms; N2 foot 245–265 ms; P2 foot 340–395 ms; N1 thigh 175–185 ms; N2 thigh 225–260 ms; P2 thigh 330–390 ms (de Tommaso et al., 2014a, 2017a, 2017b).

Thus, the LEP wave amplitudes and latencies were measured at the maximal peak in the predetermined interval.

2.8. Loreta analysis

We performed sLORETA to generate the topographical analysis of LEPs (SAKA 2011 version) (Pascual-Marqui, 2002; Fuchs et al., 2002; Jurcak et al., 2007). Previous experimental studies have supported the usefulness and validity of sLORETA in localizing generators of scalp-recorded potentials, including those related to pain processing and modulation (Vecchio et al., 2018; Gentile et al., 2020). LEPs in the −100 to 800 ms intervals were subjected to the sLORETA analysis. We applied a randomization procedure for the statistical non-parametric maps (SnPM), with 5000 randomizations according to the LORETA software.

2.9. Statistical analyses

We compared the demographic data, pain rating and sensation, and LEP latencies and amplitudes on single channels (Cz for N2 and P2 and T3 for N1) among the groups using the one-way analysis of variance (ANOVA), employed in the SPSS IBM software 21. Moreover, we evaluated the correlation between the amplitudes of major LEP waves recorded on the same channels, clinical variables (FIQ, WPI, SAS, and SDS), IENFD, and subjective pain rating (VAS) using the Pearson correlation test.

We intended to confirm or reject the hypothesis that multichannel LEP amplitude and topography could vary in patients with FM and different IENFD-no denervation, proximal denervation, proximal and distal denervation, and to outline possible differences with controls. Thus, we employed an exploratory analysis using ANOVA included in the Letswave Matlab Tool, which computes the point-by-point F- and p-values. In this ANOVA design, the amplitudes and groups were the variables and factors, respectively. The groups introduced in the analysis were as follows: FM group as a whole, healthy subjects group, FM subgroups based on IENFD (normal IEFND-FMN, proximal denervation-FMP, proximal and distal denervation- FMD).

To evaluate the habituation phenomenon in multichannel LEPs, we averaged the first and last response series. The I° series was the average of the first 10 artifact– free single potentials. In contrast, the III° series was the average of the last 10 responses. We implemented an ANOVA with diagnosis (C vs. FM) and habituation (amplitude of I° vs. III° series) as the factors. Moreover, we considered factor analysis diagnosis × habituation. We also considered the similar factor analysis to compare FM subgroups with different patterns of skin denervation.

For the sLORETA analysis, statistical differences between the groups' conditions were computed as images of voxel-by-voxel t-values. We performed a t-test for the unpaired groups, considering the single comparisons between FMN, FMD, and FMP, and the localization of the differences in cortical activity were based on the standardized electric current density, and resulted in three-dimensional t-score images. In these images, we identified cortical voxels with statistically significant differences using a non-parametric approach, with a 5% probability level threshold determined by 5000 randomizations (Pascual-Marqui, 2002). In addition, we implemented a randomization procedure to control for type I errors arising from multiple comparisons (Nichols and Holmes, 2002; Alonso et al., 2015). To test the hypothesis that LEP cortical sources were correlated with IENFD, and/or to the clinical features and psychopathological factors, we evaluated the correlation of the sLORETA matrix in the statistically relevant interval with the IENFD, FIW, WPI, SAS, and SDS, using the regression analysis included in the sLORETA software.

3. Results

Table 1 summarizes the demographic data, clinical data, and skin biopsy results.

We evaluated 22, 18, and seven patients with FM presenting with FMP, FMN, and FMD, respectively (Table 1).

Demographic and clinical data were similar between the groups. Women were prevalent in all groups (chi square 3.66, p 0.3). All patients confirmed the avoidance of analgesic use, 24 h preceding the recording session.

All patients presented with normal NCS parameters (Vecchio et al., 2020). (FMN: sural nerve amplitude and NCV: 10.21 ± 2.2 uV, 50.2 ± 10.2 m/sec; peroneal nerve amplitude and NCV 6.5 ± 2. 2 mV; 48.8 + 3.5 m/sec; FMP sural nerve 9.9 ± 4.6 uV, 49.9 + 5.5 m/sec; peroneal nerve 6.8 ± 2.6 mV, 49.8 ± 3.4 m/sec; FMD sural nerve 9.9 ± 3.4 uV; 48.9 ± 3.3 m/sec; peroneal nerve 7.1 ± 2.mV, 50.1 ± 2. 3 m/sec.)

3.1. Laser evoked potentials

3.1.1. Pain threshold and rating

The pain threshold was similar among the groups and not significant different from controls (Thigh 7.3 ± 0.1 W in controls, 7.1 ± 0.2 W in FMD; 7.2 ± 0.11 W in FMN; 7 ± 0.13 W in FMN; Foot 6.9 ± 0.2 W in controls, 6.8 ± 0.14 W in FMD; 6.7 ± 0.12 W in FMN; 6.89 ± 0.12 W in FMN). The pain rating increased in all FM groups at the foot and thigh levels, compared to the controls (Table 2).

3.1.2. Latencies, amplitudes and habituation on single channels

The LEP latencies were similar between the patients and controls and among the FM groups. The amplitudes evaluated on single channels, Cz for N2 and P2 and T3 for N1, demonstrated a significant reduction of P2 elicited by the foot in FMD (6.22 uV) and FMP (4.67 uV), compared to the controls (9.26 uV).

(Table 2). However, the N2P2 amplitude values were not different among groups (Table 1S). The habituation index (Vecchio et al, 2020) was reduced in FM patients, with significance between FMP and FMD and controls (Table 1S). The P2 wave amplitude obtained from the stimulation of the thigh was positively correlated with anxiety–SAS-scores (Pearson correlation 0.318, $p < 0.01$) and WPI (0.29, $p < 0.05$). However, other correlations were statistically insignificant.

3.2. Topographical analysis

3.2.1. Thigh stimulation

The N1 amplitude was similar among the groups, despite reduced representation of the topographical map in the FMP and FMN groups. N2 was slightly reduced in those with FMP and FMN on the central electrodes, compared to the controls, and the comparison was significant for FMP (Figs. 1–3).

The P2 wave was also reduced in patients with FMD and FMP, compared to the controls. Despite those with FMN revealing a P2 wave considerably similar to the controls, there was no difference between those with FMP and FMD (Fig. 3).

3.2.2. Foot stimulation

The N1 wave and N2 waves were similar between the controls and patients.

Patients with FMP and FMD presented with a reduced P2 amplitude in the central parietal regions than the controls. In addition, in the FM group, patients with a distal denervation demonstrated a reduced P2 amplitude than those with normal skin biopsy.

(Fig. 3)

3.3. Habituation

3.3.1. Thigh

The habituation of the N2 component was not statistically different among FM groups and controls. The P2 component displayed reduced habituation in all patients with FM, compared to the controls. Patients with normal skin innervation displayed increased P2 habituation than other FM groups (Fig. 4; Fig. 1S).

3.3.2. Foot

The P2 component habituation was slightly and insignificantly reduced in patients with respect to the controls. However, patients with distal denervation displayed potentiation rather an inhibition of the P2 component. Those with FMP and FMD displayed reduced P2 habituation than patients with normal innervation (Fig. 1S).

3.4. sLORETA analysis

The statistical comparison of cortical sources, detected significant results among groups in the time frame 330–360 msec for the thigh and 335–370 msec for the foot stimulation.

3.4.1. Thigh

The sLORETA analysis showed increased activation of Broadman area 24 (Talaraich coordinates z 5, x 29, y-1) and 32 (z 5, x 34, y-6),

Table 2
Mean. DS- standard errors - and 95% Confidence Intervals of LEP latencies and amplitudes in 15 controls (C). 18 FM patients with normal skin biopsy (FMN). 22 FM patients with proximal denervation (FMP), 7 FM patients with proximal and distal denervation (FMD). The N2 and P2 waves were evaluated on the Cz channel. the N1 wave on the T3 channel *: results of Bonferroni test.

Foot N1	Latency (msec) Mean	DS	95%		Amplitude (uV) Mean	DS	95%		VAS (0–100) Mean	DS	95%	
			Lower	Upper							Lower	Upper
C	190	0.005	175	202	3	0.6	3.5	0.6	49.1	2.3	37	69
FMD	182	0.008	164	201	3.2	0.8	4	1	58.5*	2.1	36.4	80.6
FMN	178	0.004	169	191	4	0.5	4.5	0.9	55*	2.2	32	78
FMP	181	0.002	174	186	3.9	0.25	3.9	0.89	53*	2.3	28	80.6
ANOVA (DF3)												
F									4.33			
P									0.001			
Bonferroni									*C vs FMD, FMN,FMP p 0.043			
N2												
C	242	0.019	204	281	6.82	1.1	7.9	2.8				
FMD	265	0.023	213	318	5.26	1.6	7.4	0.8				
FMN	273	0.016	240	304	4.09	0.8	6.	0.9				
FMP	268	0.007	252	283	4.77	0.46	6.2	1.2				
P2												
C	395	0.01	356	434	9.26	1.2	10.1	1.2				
FMD	404	0.02	351	457	4.67*	1.5	5	1.5				
FMN	406	0.02	374	438	8.70	0.9	9	0.9				
FMP	398	0.01	383	414	6.22*	4.4	7.1	4.4				
ANOVA (DF3)					4.23							
F					0.01							
P					*C vs FMD p.032; * C vs FMP p 0.048							
Bonferroni												
Thigh												
N1												
C	191	0.005	181	202	6.4	1.1	6.8	1.	48.9	2.1	28	65.2
FMD	189	0.008	172	205	4.1	1.5	6	1.3	59.2*	2.4	32	86.5
FMN	180	0.005	170	191	4.5	9.	5.2	1.8	52.3*	2.3	22	75
FMP	184	0.004	175	192	5.1	0.8	6	1.6	55.2*	2.1	32	79.4
ANOVA												
DF (3)												
F									2.89			
P									0.045			
Bonferroni									*C vs FMD. FMN.FMP p 0.032			
N2												
C	201	0.015	190	256	8.48	1.4	10.6	1.1				
FMD	203	0.018	157	254	5.58	2.2	7.68	2.8				
FMN	200	0.014	170	222	7.99	1.4	8.2	1.4				
FMP	206	0.011	181	223	5.7	1.1	6.6	1.8				
P2												
C	356	0.01	326	386	10.4	1.4	10.4	1.4				
FMD	340	0.023	294	386	8.3	2.1	8.3	2.1				
FMN	339	0.014	310	367	10.8	1.3	10.8	1.3				
FMP	346	0.012	323	370	8.4	1.1	8.4	1.1				

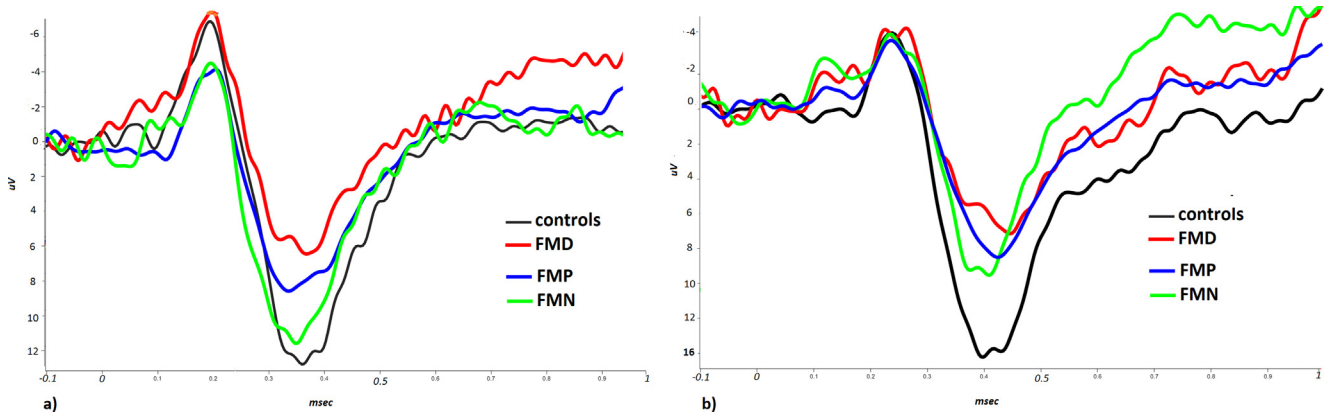

Fig. 1. Grand average of laser evoked potentials recorded from a) the right thigh and b) the right foot in controls and patients with fibromyalgia, divided in accord with skin biopsy. FMD-patients with distal and proximal intraepidermal nerve fibers density (IENDF)-reduction; FMN: normal IENFD; and FMP: proximal IENFD reduction.

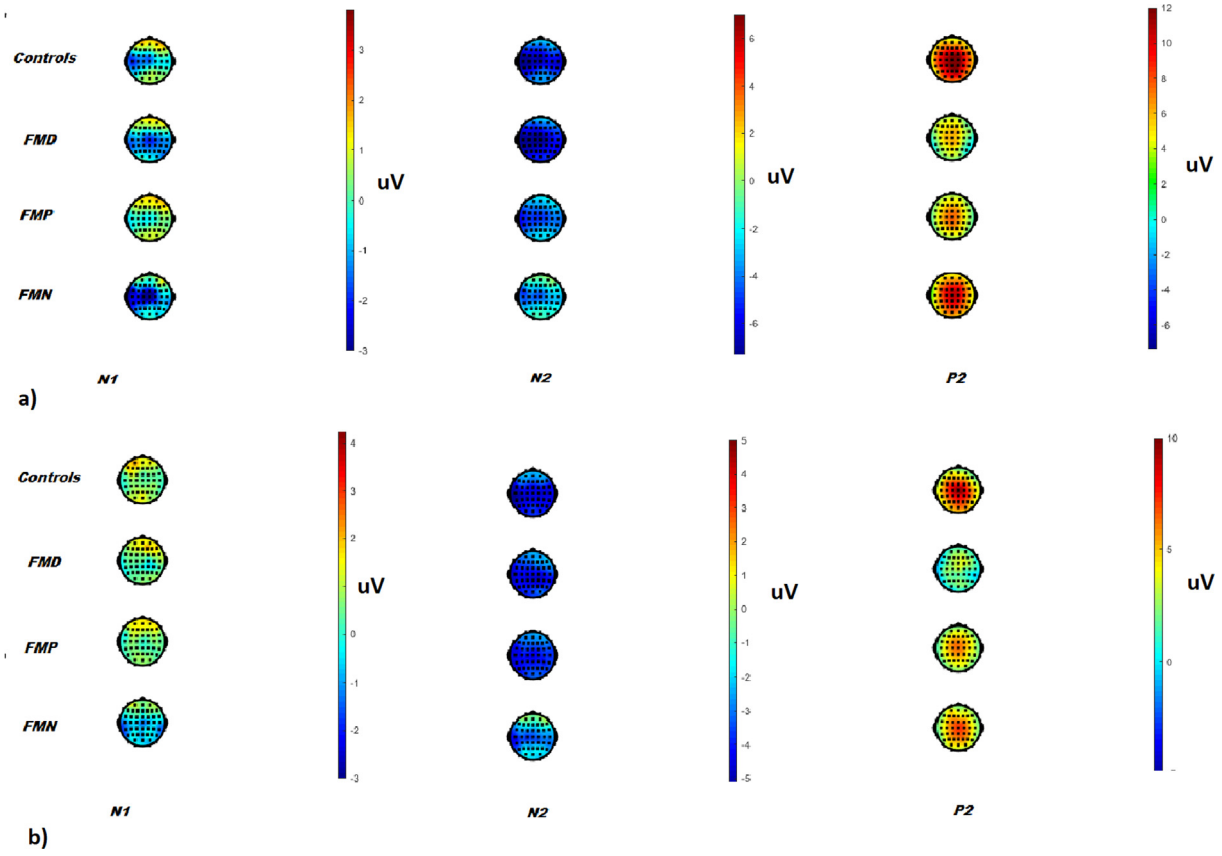

Fig. 2. Topographic maps depicting laser evoked potentials LEP waves from a) thigh stimulation and b) foot stimulation in Fibromyalgia subgroups and controls. The representation of P2 wave on the central regions seemed reduced in FMP and FMD patients, while it was similar to controls in FMN group. Also the N2 seemed less represented on bilateral temporal-parietal regions in FMN and FMP groups. FMD-patients with distal and proximal intraepidermal nerve fibers density (IENDF)-reduction; FMN: normal IENFD; and FMP: proximal IENFD reduction.

corresponding to anterior cingulate gyrus, in FMP patients compared to controls (Fig. 5a) (Table 2S). The comparison between FMD and controls and FMN and controls did not reach statistical significance. The comparison among FM groups was not significant

3.4.2. Foot

Patients included in the FMP group revealed increased activation of the anterior and posterior cingulate (area 31 – Talaraich coordinates z-15, x– 32, y38 - and area 24) than the controls (Fig. 5b). Patients with FMD displayed increased activation in the anterior cingulate (area 32) (Fig. 5c). Moreover, those without an evidence of denervation demonstrated significant sLORETA changes in the prefrontal lobe, areas 10 (coordinates z-40, x49, y 11) and 11, dorsolateral prefrontal cortex (area 46), inferior frontal gyrus (area 47), and anterior cingulate (area 24) than the controls (Fig. 5d). The comparison between the FM groups was insignificant.

3.5. Correlation between sLoreta signal, epidermal nerve density, and clinical features

We observed no correlation between the IENFD and cortical sources, corresponding to the late LEP component evoked by thigh stimulation. FM invalidity scores corresponded to a significant decrease in the activity of the anterior cingulate area 31 (Fig. 6a). Anxiety was associated with increased activity in the cingulate cortex area 30 (Fig. 6b). Furthermore, the depression score was correlated to a reduced activity of area 32 (Fig. 6c). The diffusion of pain correlated with the activation of the right insula in area 13 (Fig. 6d) (Table 2S). However, the laser pain perception did not correlate with the dipolar cortical sources activated by foot and thigh stimulation.

4. Discussion

In summary, our findings based on a multichannel study of LEPs demonstrated reduced amplitude of P2 in patients with FM, predominantly those presenting with FMD, despite no strict topographic correspondence between the amplitude abnormalities and the prevalent site of denervation (proximal and/or distal). Reduced P2 habituation prevailed in patients with epidermal skin density reduction. The laser stimulus resulted in an increased activation of the cingulate cortex and prefrontal cortex in FM groups, compared to the controls. The severity of proximal denervation did not reveal a linear correlation with the strength of late wave dipole activation. However, we observed a correlation with the clinical variables, such as anxiety, depression, FM invalidity, and pain diffusion.

4.1. LEPs topographic analysis in FM with varied epidermal nerves density

The pattern of reduced LEP amplitude with normal latency prevailed in patients with FM, in accordance with other groups (Evdokimov et al., 2019). Previous studies have reported on a variability in the N2P2 amplitude among patient groups and a lack of linear correlation with IENFD (de Tommaso et al., 2014a, 2014b; Vecchio et al., 2020). Fasolino et al. (2020) recently mentioned that

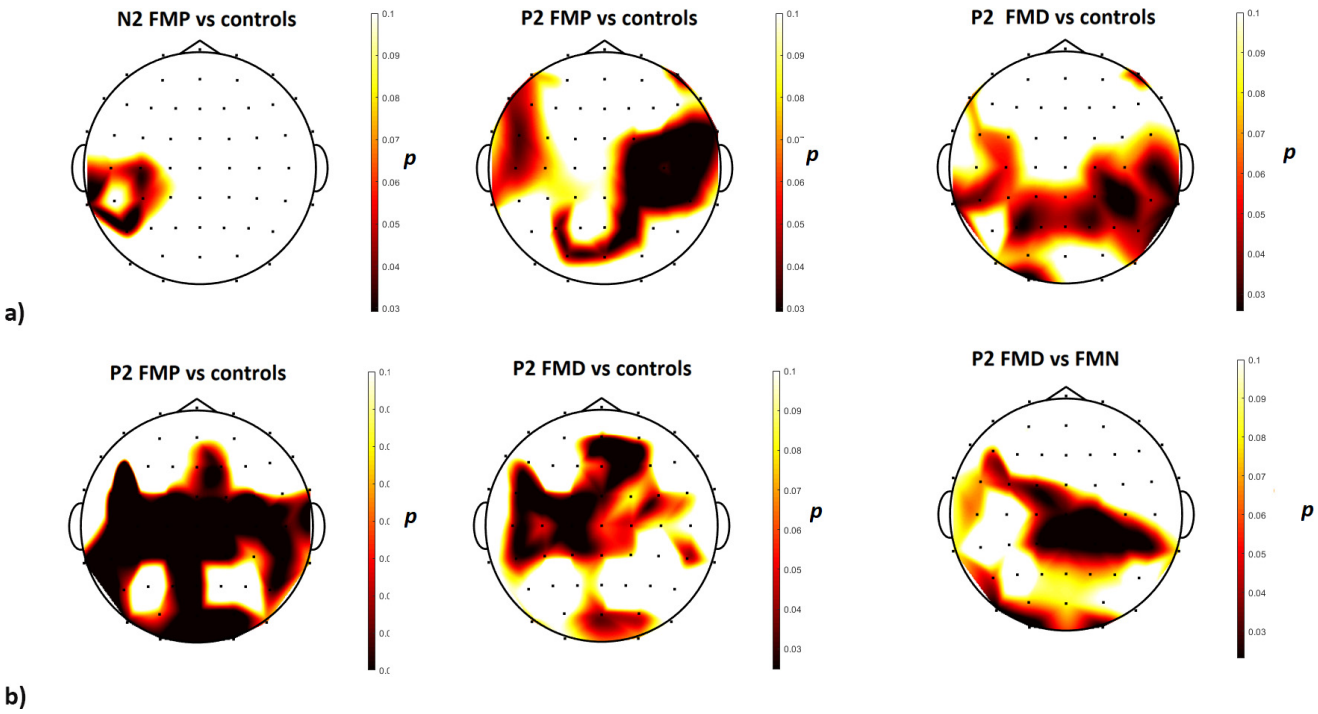

Fig. 3. Statistic probability maps computed on 65 scalp electrodes among FM subgroups and controls for a) thigh stimulation and b) foot stimulation. The maps report the results of analysis of variance contrasts between the groups, considering the maximal peak in the N2 and P2 time intervals and the groups as variables and factors, respectively. The black and brown colors represent a significant result. We can observe a statistical difference of N2 wave obtained at the thigh, located in a limited left temporal region between FMP patients and controls. The statistical differences of P2 wave amplitude between groups, were present for the thigh and the foot stimulation. The regions of such differences, were mainly located around the vertex, sometimes without involving the Cz electrode, as at the thigh for the comparison between FMP and FMD vs controls. FMD-patients with distal and proximal intraepidermal nerve fibers density (IENDF)-reduction; FMN: normal IENFD; and FMP: proximal IENFD reduction.

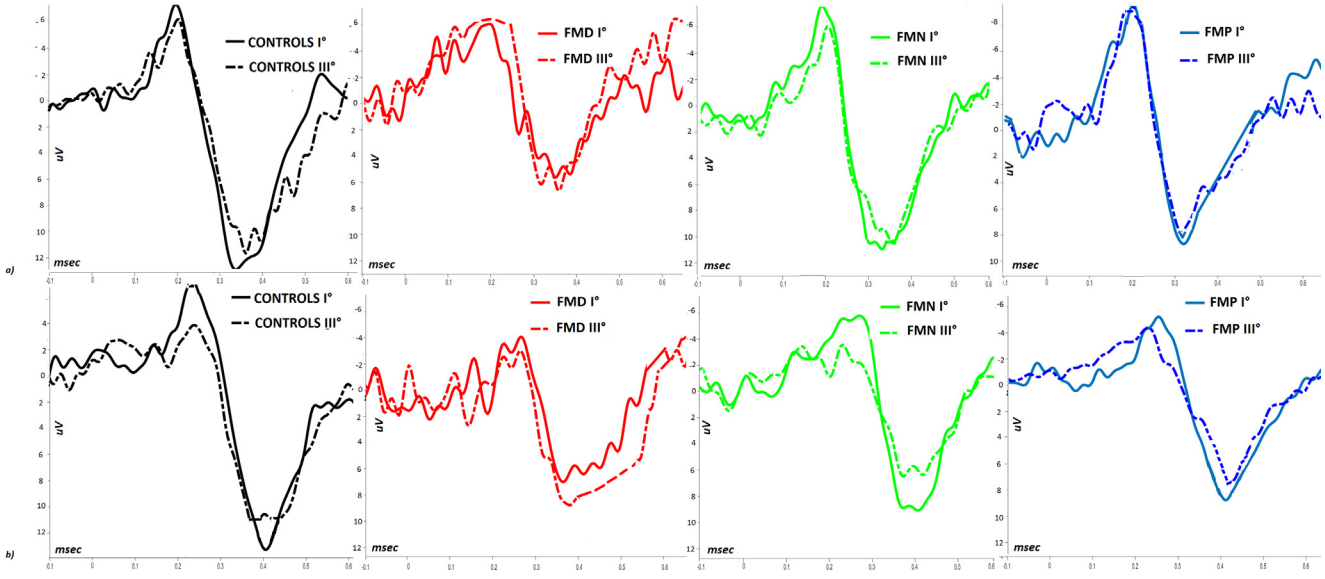

Fig. 4. Grand Average of the I° and III° series of 10 stimuli in FM subgroups and controls for a) thigh and b) foot stimulation. In controls, a reduction of P2 and N2 waves is present in the III repetition, represented with dotted line. In FMD, the P2 augmented at the third repetition, and slightly decreased at the thigh and foot in FMP. FMD-patients with distal and proximal intraepidermal nerve fibers density (IENDF)-reduction; FMN: normal IENFD; and FMP: proximal IENFD reduction.

such patients with prevalent distal denervation did not display N2P2 amplitude reduction.

The employment of multichannel LEPs and the topographical detection of statistical changes between those with FM and controls could have emphasized the tendency to amplitude reduction of the late vertex complex. Recent studies employing the standard few channel montage considered the total amplitude of the N2P2 vertex complex (Van Assche et al., 2020), which was substantially normal in patients with FM than their age-matched controls. We observed reduced P2 component in the amplitude on several channels, sometimes without involving the Cz electrode, as for the thigh stimulation. However, the N2 wave elicited from the foot was sim-

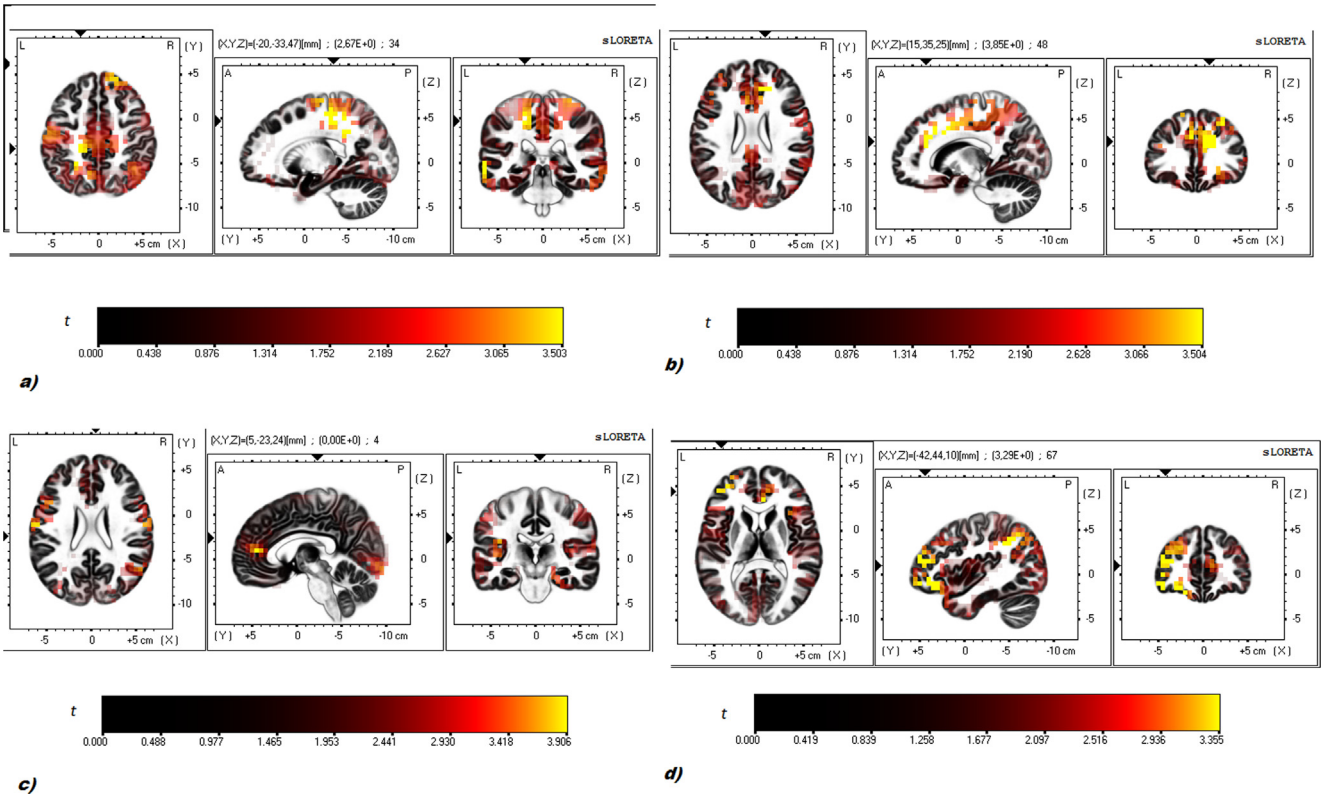

Fig. 5. Statistical comparison of sLORETA (standardized low-resolution brain electromagnetic tomography) matrix computed in the time frame, corresponding to the P2 component elicited by the right thigh (a) and the right foot (b, c, and d). For the thigh, the sLORETA voxels express the statistically analyzed results of P2 sources between the FMP group and controls. The map expresses the maximal difference in yellow, corresponding to anterior cingulate Brodmann areas 24 and 32 (a). For the foot, the sLORETA voxels express the statistically analyzed results of P2 sources compared among FMP (b), FMD (c), FMN (d), and controls. Patients included in the FMP group reveal increased activation of the anterior and posterior cingulate (areas 31 and 24) than the controls (b). Similar results have been observed in those with FMD (c). Patients without an evidence of denervation reveal significant sLORETA changes in the prefrontal lobe, areas 10 and 11, dorso lateral prefrontal cortex (area 46), inferior frontal gyrus (area 47), and anterior cingulate (area 24) than controls (d) (Table 2S). The maps denote the maximal difference in yellow. FMD-patients with distal and proximal IntraEpidermal Nerve Fibers Density (IENDF)-reduction; FMN: normal IENFD; sLORETA, standardized low-resolution brain electromagnetic tomography; and FMP: proximal IENFD reduction.

ilar among the groups. Therefore, in some FM cases, the total amplitude of the vertex complex would be normal at least on the Cz derivation.

There was no strict correspondence between the late wave amplitude reduction and the site involved in the denervation phenomenon. For example, patients with prevalent proximal IENFD reduction displayed low amplitude of P2 component at the foot than the healthy subjects. Moreover, patients with FM without proximal and/or distal denervation had normal N2 and P2 amplitudes for thigh stimulation, consistent with the normal function of peripheral A-delta afferents. The P2 amplitude in the aforementioned patients appeared reduced for foot stimulation, compared to the controls. However, the amplitude was higher at the foot than that in patients with FM and distal neuropathy. Despite a modulation of the P2 component among those with FM and different IENFD, according to a tendency towards decreased amplitude with decreased skin innervation, there was no correspondence between the site of low LEP amplitude and that with prevalent skin denervation, and a reduction in LEP in patients with FMN. First, the restricted number of cases in single FM groups invalidated the statistical significance, particularly for those with FMD. Mild and initial damage to the A-delta fibers may be a cause of LEP reduction in patients with normal skin biopsy parameters. Moreover, the IENFD is supposed to test mainly the unmyelinated (C) fibers, while LEPs test the function of the Adelta fibers. The use of analgesics could also exert an impact on LEP amplitudes. Moreover, analgesics could exert a long-lasting inhibitory effect on the late P2 wave. However, we recommended avoiding their use in the last 24 h (Truini et al., 2010). Thus, pharmacological treatment may represent a limit for LEP examination in patients with FM in routine clinical practice (Schaffler et al., 2018). Psychopathological factors play a role in modulating the LEP amplitude. For example, depression causes a late LEP wave amplitude reduction (Terhaar et al., 2011). Our patients did not meet the criteria of major depression. Nonetheless, depressive traits were present in several patients.

The presence of normal N1 amplitude also contradicts the hypothesis of a classical pattern of peripheral neuropathy (Casanova-Molla et al., 2011). This in turn highlights FM as a complex disorder, different from small fiber neuropathy. Present data confirm heterogeneous phenotypes among FM patients, based on a mixture of PNS and CNS dysfunctional factors (de Tommaso et al., 2014b; Vecchio et al., 2020).

4.2. LEP habituation

We confirmed the lack of P2 habituation in all FM groups, compared to the healthy subjects (de Tommaso et al., 2011; Vecchio et al., 2020). Reduced habituation of the P2 component was modulated by the degree of peripheral denervation, in accordance with previous studies (Vecchio et al., 2020). In fact, the peripheral dysfunction of a-delta afferents could determine an initial reduction of cortical activity with a progressive recovery in the course of repetitive stimulation. This phenomenon was similar to that described in migraine, wherein the decrease in cortical pre-activation level supposedly reduces habituation (Coppola et al., 2009). The P2 of patients with normal IENFD habituated more than patients with

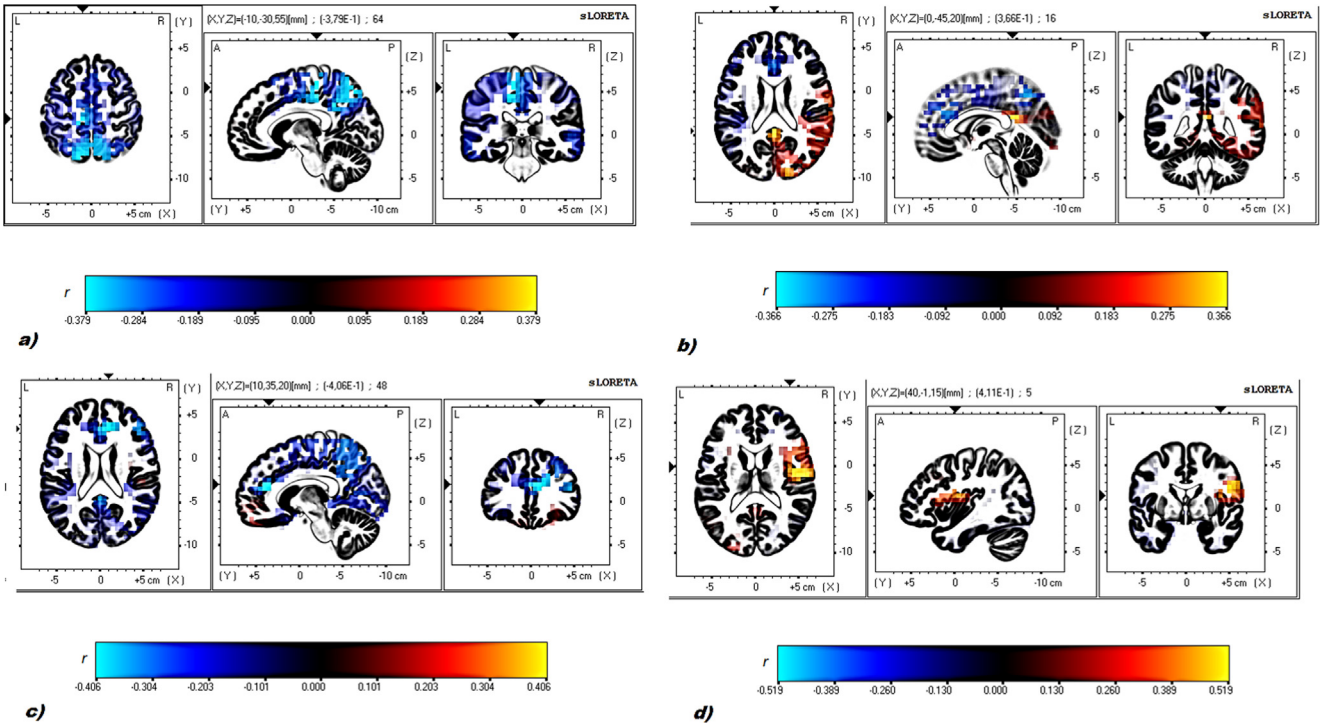

Fig. 6. sLORETA (standardized low-resolution brain electromagnetic tomography matrix) in the time frame of the P2 component elicited by the thigh, expressing the value of linear correlation– Pearson correlation- in 47 patients with FM (Fibromyalgia). Light blue colors express negative correlation, yellow colors positive correlation. Fibromyalgia invalidity scores correspond to a significant decrease in the activity of anterior cingulate area 31 (a). Anxiety scores are associated to increased activity in cingulate cortex area 30 (b). The depression scores are associated to a reduced activity of area 32 (c). The diffusion of pain is correlated with the activation of the right insula, area 13 (d) (Table 2S).

FMD at all stimulated sites. Moreover, those with distal denervation demonstrated a complete loss of habituation or potentiation in the course of repetitive stimulation, particularly at the distal sites of stimulation. In other words, reduced habituation is a mechanism of compensation for peripheral afferent loss, present in painful neuropathies with abnormal central pain processing (Hüllemann et al. 2017).

However, this mechanism could not completely restore LEP amplitudes in some patients with FM, owing to the possible coexistence of long-lasting analgesic effects, psychopathological factors, and the severity of peripheral denervation.

4.3. sLoreta analysis

Despite the tendency for the reduced amplitude of the P2 wave, some dipolar sources activated in the P2 time frame increased in patients with FMP at all stimulated sites, excluding the FMN and FMD groups for the stimulation of the thigh. However, we observed similar trends in these patients. The sLoreta analysis was previously applied to LEPs, and the activation of the major cortical sources confirmed what is described with single dipole analysis (Calabrò et al., 2016; Brown et al., 2008). In patients with FM, acupuncture modulated the major cortical sources of the N2P2 complex, located in the anterior cingulate, insula, and inferior operculum (de Tommaso et al., 2014a).

We observed increased activation of the cortical sources of LEPs generated from the cingulate cortex, both in the anterior and posterior portions (Bradley et al., 2017; Mahmutoglu et al. 2021). The small fiber loss did not influence the modality of cortical source activation, as we did not observe differences among patients with varied IENFD. Moreover, in patients with small fibers loss, the decrease in amplitude of late LEP could be associated with a functional readjustment within the major cortical sources, such as those in the cingulate cortex, with persistent hyper-activation. Furthermore, a reduced habituation of the P2 component could be subtended by the compensatory hyperactivity of the cingulate cortex.

In patients with FMN, the orbitofrontal and dorsolateral prefrontal cortex sLoreta matrices displayed higher values than the controls. These cortical areas have not been previously attributed to the generation of LEPs. However, they are involved in the processing of pain stimuli (Rolls et al., 2003) and pain modulation (Lorenz et al., 2003; Dunckley et al., 2005).

Most studies employing functional neuroimaging in FM have described altered cortical activation and connectivity in regions devoted to pain processing and descending control (Staud, 2011). This is the first study to compare the activation of cortical regions related to pain stimuli processing in patients with different peripheral nerve involvement. Based on our findings, peripheral dysfunction does not exert an influence on the central processing of pain. Increased activation of the anterior and posterior cingulate and prefrontal areas devoted to pain processing are present in patients with FM, independent of the degree of peripheral A-delta fiber loss.

While the intra epidermal nerves fiber immunostained by the polyclonal anti-protein gene product 9.5 (PGP-9.5) explores several subpopulations of fibers, LEPs merely inform the functional status of a small subpopulation, i.e, the AMH type II nociceptors. This could justify the lack of correlation between LEP amplitudes and IENFD. Moreover, in peripheral neuropathy, a biopsy-verified decrease in thin fiber density was correlated with the LEP amplitude decrease (Casanova-Molla et al., 2011). In accordance with the lack of correspondence between the degree of peripheral denervation and LEP amplitude (Vecchio et al, 2020), we did not observe a linear relationship between IENFD and the strength of cortical source activation.

Therefore, the cortical response to nociceptive stimuli could be influenced by different factors, which extend beyond the number of peripheral afferents. The correspondence observed in painful

neuropathies between the degree of skin afferent loss and LEP amplitudes (Casanova-Molla et al., 2011) was not evident in those with FM. The aforementioned complex modality of a central elaboration of peripheral input serves as the neurophysiological counterpart of the clinical phenotype, not resembling the clinical picture of typical small fiber neuropathy (Fasolino et al., 2020). Moreover, the depression scores corresponded to reduced activation of the anterior cingulate, as well as the global index of disability. This in turn confirmed our hypothesis that the modulation of LEPs in patients with FM was not directly correlated to the number of A-delta afferents, but was influenced by psychopathological factors and the clinical expression of pain.

Patients with anxiety tracts displayed prevalent posterior cingulate activation. Previous studies on the connections among the default mode network in patients with FM mentioned that anxiety influenced the connection strength in the ventromedial prefrontal and posterior cingulate cortex (van Ettinger-Veenstra et al., 2020). The posterior cingulate cortex is one of the generators of late LEPs (Bradley et al., 2017; Mahmutoglu et al., 2021), and its prevalent activation in anxious patients with FM may be attributed to its involvement in the arousal state (Leech and Sharp, 2014). Pain diffusion corresponds to an increased activity of the omolateral insula. The posterior insula is one of the sources of late positive LEPs (Mahmutoglu et al., 2021), and its role in pain intensity coding has been largely demonstrated (Coghill et al., 1999). The relationships between sLORETA cortical sources and clinical variables partially corresponded with the correlations we observed, considering the LEP amplitude evaluated on a single channel. This finding is reasonable because a dipolar source analysis arises from multichannel recordings.

4.4. Study limitation

The sample size was small for the single FM subgroups, particularly those with FMD. The effect of symptomatic drugs on LEPs could not be excluded. Our results are in favor of a multifactorial modulation of LEP amplitude and cortical generators, which would be better examined with a multiregression model analysis. Unfortunately, our statistical program did not support this opportunity. In addition, we should have considered the limits of the ERP source analysis (Asadzadeh et al., 2020). In fact, the possibility of showing "fake" sources is not excluded in multi dipolar LORETA analysis. This could explain the involvement of some areas, such as the prefrontal cortex or the posterior cingulate cortex, whose activation has rarely be found with laser stimulation.

The lack of correspondence between the cortical generators of LEPs and skin biopsy could be partly because of the different types of fibers evaluated using the two methods (Vecchio et al, 2020).

5. Conclusions

The present multichannel LEP study confirmed a tendency toward a modulation of late P2 wave, in accordance with the degree of peripheral afferents loss, without a direct relationship with IENFD. We presently confirm that FM patients did not resemble a classical neurophysiological model of neuropathic pain, with a global reduction of cortical waves obtained from the sites of nociceptive afferents damage (Haanpää et al., 2011). The clinical relevance of such P2 abnormality in single patients, should be considered in light of different factors. Psychopathological features and pain somatic diffusion, supposedly play a role in the modulation of late LEP generators. The use of symptomatic drugs and the possible existence of A-delta afferent dysfunction at the initial stage cannot be excluded. Reduced habituation was a mechanism of partial restoration of late LEP waves, consistent with the degree of peripheral afferent loss. The activation of cortical regions with a key role in pain processing and modulation was evident in almost all patients with FM, independent of peripheral small fiber deficiency. The existence of different FM phenotypes, characterized by a different mixture of peripheral and central factors involved in pain processing and clinical features, is the major impression emerging from these data. Moreover, multichannel LEP did not resemble a pattern of peripheral neuropathy in such patients. In contrast, they reflected the complexity of the disease.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2022.01.001.

References

Alonso JF, Romero S, Mañanas MÁ, Rojas M, Riba J, Barbanoj MJ. Evaluation of multiple comparison correction procedures in drug assessment studies using LORETA maps. Med Biol Eng Comput 2015;53(10):1011–23.

Asadzadeh S, Yousefi Rezaii T, Beheshti S, Delpak A, Meshgini S. A systematic review of EEG source localization techniques and their applications on diagnosis of brain abnormalities. J Neurosci Methods 2020;339:108740. https://doi.org/10.1016/j.jneumeth.2020.108740.

Bidari A, Hassanzadeh M, Mohabat M-F, Talachian E, Khoei EM. Validation of a Persian version of the Fibromyalgia Impact Questionnaire (FIQ-P). Rheumatol Int 2014;34(2):181–9.

Bradley C, Bastuji H, Garcia-Larrea L. Evidence-based source modeling of nociceptive cortical responses: A direct comparison of scalp and intracranial activity in humans. Hum Brain Mapp 2017;38(12):6083–95.

Brown CA, Seymour B, Boyle Y, El-Deredy W, Jones AKP. Modulation of pain ratings by expectation and uncertainty: Behavioral characteristics and anticipatory neural correlates. Pain 2008;135:240–50.

Calabrò RS, Naro A, Manuli A, Leo A, De Luca R, Lo Buono V, et al. Pain perception in patients with chronic disorders of consciousness: What can limbic system tell us? Clin Neurophysiol 2016;128(3):454–62.

Casanova-Molla J, Grau-Junyent JM, Morales M, Valls-Solé J. On the relationship between nociceptive evoked potentials and intraepidermal nerve fiber density in painful sensory polyneuropathies. Pain 2011;152:410–8.

Coghill RC, Sang CN, Maisog JM, Iadarola MJ. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J Neurophysiol 1999;82(4):1934–43.

Coppola G, Pierelli F, Schoenen J. Habituation and migraine. Neurobiol Learn Mem 2009;92(2):249–59. https://doi.org/10.1016/j.nlm.2008.07.006.

de Tommaso M, Delussi M, Ricci K, D'Angelo G. Abdominal acupuncture changes cortical responses to nociceptive stimuli in fibromyalgia patients. CNS Neurosci Ther 2014a;20(6):565–7.

de Tommaso M, Federici A, Santostasi R, Calabrese R, Vecchio E, Lapadula G, et al. Laser-evoked potentials habituation in fibromyalgia. J Pain 2011;12(1):116–24.

de Tommaso M, Nolano M, Iannone F, Vecchio E, Ricci K, Lorenzo M, et al. Update on laser-evoked potential findings in fibromyalgia patients in light of clinical and skin biopsy features. J Neurol 2014b;261(3):461–72.

de Tommaso M, Ricci K, Libro G, Vecchio E, Delussi M, Montemurno A, et al. Pain Processing and Vegetative Dysfunction in Fibromyalgia: A Study by Sympathetic Skin Response and Laser Evoked Potentials. Pain Res Treat 2017a;2017:1–12.

de Tommaso M, Ricci K, Montemurno A, Vecchio E. Age-related changes in laser-evoked potentials following trigeminal and hand stimulation in healthy subjects. Eur J Pain 2017b;21(6):1087–97.

Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain 2008;131:1912–25.

de Tommaso M, Vecchio E. Primary headaches and trigeminal neuralgia: neuropathic pain yes or not? Evidences from neurophysiological procedures. Expert Rev Neurother 2013;13(9):1031–9.

Dunckley P, Wise RG, Aziz Q, Painter D, Brooks J, Tracey I, Chang L. Cortical processing of visceral and somatic stimulation: differentiating pain intensity from unpleasantness. Neuroscience 2005;133(2):533–42.
Evdokimov D, Frank J, Klitsch A, Unterecker S, Warrings B, Serra J, et al. Reduction of skin innervation is associated with a severe fibromyalgia phenotype. Ann Neurol 2019;86(4):504–16.
Fasolino A, Di Stefano G, Leone C, Galosi E, Gioia C, Lucchino B, et al. Small-fibre pathology has no impact on somatosensory system function in patients with fibromyalgia. Pain 2020;161(10):2385–93.
Fuchs M, Kastner J, Wagner M, Hawes S, Ebersole JS. A standardized boundary element method volume conductor model. Clin Neurophysiol. 2002;113 (5):702–12.
Gentile E, Ricci K, Vecchio E, Libro G, Delussi M, Casas-Barragàn A, et al. A Simple Pattern of Movement is not Able to Inhibit Experimental Pain in FM Patients and Controls: an sLORETA Study. Brain Sci 2020;10(3):190. https://doi.org/10.3390/brainsci10030190.
Gibson SJ, Littlejohn GO, Gorman MM, Helme RD, Granges G. Altered heat pain thresholds and cerebral event-related potentials following painful CO2 laser stimulation in subjects with fibromyalgia syndrome. Pain 1994;58:185–93.
Grayston R, Czanner G, Elhadd K, Goebel A, Frank B, Üçeyler N, et al. A systematic review and meta-analysis of the prevalence of small fiber pathology in fibromyalgia: Implications for a new paradigm in fibromyalgia etiopathogenesis. Semin Arthritis Rheum 2019;48(5):933–40.
Haanpää M, Attal N, Backonja M, Baron R, Bennett M, Bouhassira D, et al. NeuPSIG guidelines on neuropathic pain assessment. Pain 2011;152(1):14–27.
Hüllemann P, von der Brelie C, Manthey G, Düsterhöft J, Helmers AK, Synowitz M, Baron R. Reduced laser-evoked potential habituation detects abnormal central pain processing in painful radiculopathy patients. Eur J Pain 2017;21 (5):918–26.
Jurcak V, Tsuzuki D, Dan I. 10/20, 10/10, and 10/5 systems revisited: Their validity as relative head-surface-based positioning systems. NeuroImage 2007;34 (4):1600–11.
Kimura J. Electrodiagnosis in Diseases of Nerve and Muscle. Oxford University Press; 2013.
Lauria G, Hsieh ST, Johansson O, Kennedy WR, Leger JM, Mellgren SI, et al. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the Use of Skin Biopsy in the Diagnosis of Small Fibre Neuropathy. J Periph Nerv Syst 2010;15:79–92.
Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. Brain 2014;137:12–32.
Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 2003;126:1079–91.
Mahmutoglu MA, Baumgärtner U, Rupp A. Posterior insular activity contributes to the late laser-evoked potential component in EEG recordings. Clin Neurophysiol 2021;132(3):770–81.
Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: A primer with examples. Hum Brain Map 2002;15(1):1–25.
Pascual-Marqui RD. Standardized low-resolution brain electromagnetic tomography (sLORETA): Technical details. Methods Find Exp Clin Pharmacol 2002;24:5–12.
Rolls ET, O'Doherty J, Kringelbach ML, Francis S, Bowtell R, McGlone F. Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. Cereb Cortex 2003;13:308–17.
Schaffler K, Yassen A, Reeh P, Passier P. A Randomized, Double-Blind, Placebo- and Active Comparator-Controlled Phase I Study of Analgesic/Antihyperalgesic Properties of ASP8477, a Fatty Acid Amide Hydrolase Inhibitor, in Healthy Female Subjects. Pain Med 2018;19:1206–18.
Staud R. Brain imaging in fibromyalgia syndrome. Clin Exp Rheumatol 2011;29(6 Suppl 69):S109–17.
Terhaar J, Viola FC, Franz M, Berger S, Bär KJ, Weiss T. Differential processing of laser stimuli by Aδ and C fibres in major depression. Pain 2011;152:1796–802.
Truini A, Panuccio G, Galeotti F, Maluccio MR, Sartucci F, Avoli M, Cruccu G. Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. Eur J Pain 2010;14:222–5. https://doi.org/10.1016/j.ejpain.2009.05.001.
Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, et al. Small fibre pathology in patients with fibromyalgia syndrome. Brain 2013;136:1857–67.
Van Assche DCF, Plaghki L, Masquelier E, Hatem SM. Fibromyalgia syndrome-A laser-evoked potentials study unsupportive of small nerve fibre involvement. Eur J Pain 2020;4:448–56.
van Ettinger-Veenstra H, Boehme R, Ghafouri B, Olausson H, Wicksell RK, Gerdle B. Exploration of Functional Connectivity Changes Previously Reported in Fibromyalgia and Their Relation to Psychological Distress and Pain Measures. J Clin Med 2020;9(11):3560. https://doi.org/10.3390/jcm9113560.
Vecchio E, Lombardi R, Paolini M, Libro G, Delussi M, Ricci K, et al. Peripheral and central nervous system correlates in fibromyalgia. Eur J Pain 2020;24 (8):1537–47.
Vecchio E, Gentile E, Franco G, Ricci K, de Tommaso M. Effects of external trigeminal nerve stimulation (eTNS) on laser evoked cortical potentials (LEP): A pilot study in migraine patients and controls. Cephalalgia 2018;38(7):1245–56.
Wolfe F, Clauw DJ, Fitzcharles M-A, Goldenberg DL, Katz RS, Mease P, et al. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthr Care Res 2010;62 (5):600–10.
Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthr Rheum 1990;33(2):160–72.
Yunus MB. Editorial review: an update on central sensitivity syndromes and the issues of nosology and psychobiology. Curr Rheumatol Rev 2015;11:70–85.
Zung W. How normal is anxiety? Current Concepts. Upjohn, Durham, North Carolina, USA; 1980.
Zung W. SAS, Self-Rating Anxiety Scale. In: Guy W, editor. ECDEU assessment manual for psychopharmacology. revised edn. Rockville, MD: National Institute of Health, Psycho-pharmacology Research Branch; 1976. p. 337–40.