PERIPHERAL AND CENTRAL NERVOUS SYSTEM CORRELATES IN FIBROMYALGIA

Running head: Peripheral and central correlates of pain in fibromyalgia

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ABSTRACT

Background: Fibromyalgia (FM) is a syndrome characterized by altered pain processing at central and peripheral level, whose pathophysiologic mechanisms remain obscure. We aimed at exploring the structural changes of peripheral nociceptor measured by skin biopsy, the functional changes of central nociceptive pathway assessed by laser evoked potentials (LEP), and their correlation with clinical features and comorbidities.

Methods: Eight-one patients diagnosed with FM underwent skin biopsies with quantification of intraepidermal nerve fiber density (IENFD) at the thigh and distal leg, and LEP recording by stimulating hand, thigh and foot. Nerve conduction study (NCS), clinical features, comorbidity with migraine and mood disorders, and previous, non-active immune-mediated disorders were recorded.

Results: IENFD was reduced in 85% of patients at the thigh and in 12.3% of patients at the distal leg, whereas it was normal in 14.8% of patients. N2P2 habituation index from laser stimulation at the thigh was altered in 97.5% of patients and correlated with reduced IENFD at the thigh. LEP latencies and amplitudes did not differ among groups. No association was found between IENFD, LEP, clinical features, and comorbidities.

Conclusions: FM patients most commonly showed a mild loss of peripheral nociceptors at the thigh rather than distal small fiber neuropathy. This finding was associated with an altered habituation index and strengthened the hypothesis that central sensitization plays a key role in the pathogenesis of the disease.

Significance: Central impairment of pain processing likely underlies FM, which in most patients is associated with mild proximal small fiber pathology.

Key words: fibromyalgia, skin biopsy, small fibers pathology, laser evoked potentials, habituation

INTRODUCTION

Fibromyalgia (FM) is a chronic and disabling disease dominated by diffuse pain and several associated symptoms, such as sleep disorder, cognitive impairment, and fatigue (Wolfe et al, 2010). The clinical features included in the diagnostic criteria outline the complexity of the disease, as many could be due to dysfunction of central and/or peripheral nervous system. Brain functional analyses revealed altered pain processing at central level (Cook et al., 2004; López-Solà et al., 2017). Neurophysiological studies exploring pain pathways described an abnormal pattern of increased amplitude and reduced habituation of cortical responses (Gibson et al., 1994; de Tommaso et al., 2011). This was similar to what observed in migraine patients, in whom the prevalence of FM has been estimated to range between 10% and 37% (de Tommaso et al., 2005, 2011).

Self-sustained central sensitization has been considered a driving cause of FM (Yunus, 2007). However, in the last few years, reports describing small fibers pathology in a considerable number of patients have challenged this hypothesis. Since the first study (Üçeyler et al. 2013), others independently showed that a large percentage of patients have non-length dependent decrease of intraepidermal nerve fiber density (IENFD), which reflects the loss of the most distal nociceptors (Lauria et al, 2006) and might explain some clinical features of the syndrome such as peripheral autonomic impairment (Caro et al, 2018; Giannoccaro et al, 2014; Kosmidis et al. 2014). A non-length dependent small fiber pathology was confirmed also by corneal confocal microscopy and nociceptive evoked response studies (Evdokimov et al, 2019; Levine, 2015; Ramirez et al., 2015; de Tommaso et al., 2014). In a previous study, we tested laser evoked potentials (LEP), which specifically investigate the nociceptive pathways (de Tommaso et al., 2014), in a large group of FM patients, and observed that most of the patients had reduced habituation of late cortical responses (de Tommaso et al., 2014). LEPs reflect overall transmission in peripheral and central nociceptive pathways. LEP habituation to repeated stimuli may reflect central processing of nociceptive stimuli, in the sense of habituation (fatigue) or lack of habituation or even potentiation (sensitization) (Rankin CH et al ., 2009)

An unbalanced processing of peripheral (PNS) and central nervous system (CNS) nociceptive signaling is thought to underlie the pathophysiology of FM, even though its phenotypic heterogeneity that includes comorbidity with migraine (de Tommaso et al., 2014, 2016)

remains unanswered. The presence of severe anxiety and depression could interfere with the amplification of pain at CNS level, and many somatic symptoms frequently indicate a clinical phenotype with prevalent psychiatric rather than peripheral nervous system involvement.

To what extent CNS and PNS impairment coexist, and what is the weight of each in inducing the full-blown clinical picture is unknown. We sought to contribute in addressing this question through a study that evaluated frequency, distribution and correlation of LEP and IENFD abnormalities as proxies of CNS and PNS impairment, respectively, in a deeply phenotyped Italian cohort of FM patients.

METHODS

This was an observational study conducted at the Applied Neurophysiology and Pain Unit of Policlinico General Hospital of Bari, Italy. Patients referred from January to December 2017 were selected. Inclusion criteria were diagnosis of FM according to the 2010 American College of Rheumatology criteria (Wolfe et al., 2010) and to be aged between 18 and 75 years. Exclusion criteria were education below 8 years and any cause of PNS or 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 other than anxiety and depression disorders according to the Diagnostic and Statistical Manual of Mental Disorders V (DSM V), active malignancies or history of cancer, use of drugs acting on the CNS and chronic opioid therapy. Patients taking analgesics were instructed to avoid use 24 hours prior to LEP recording to exclude any effect on amplitudes (Truini et al., 2010). All patients signed the informed consent. The local Ethic Committee approved the study of LEP and skin biopsy for the first time in 2009 (n° 123/2009), then yearly authorized   the prosecution of data collecting and updated the informed consent.

All patients underwent focused interview and standard neurological examination, including bedside sensory testing. All associated conditions such as migraine, anxiety and depression, history of non-active arthritis and chronic thyroiditis were recorded. Migraine was diagnosed based on the International Classification of Headache Disorders criteria for migraine with aura, without aura and chronic migraine (IHS, 2018). All patients with history of inflammatory arthritis or autoimmune disease underwent rheumatologist consult to rule out an active disease at the time of FM diagnosis. Depression and anxiety were assessed by clinical history, psychiatrist reports, and the Zung Self-Rating Depression Scale (SDS) (Zung,

1965) and Anxiety Scale (SAS) (Zung, 1976). We included in the group with psychiatric comorbidity patients with SDS ≥50 and SAS ≥45. Patients were required to fill the fibromyalgia-linked invalidity questionnaire (FIQ) (Bidari et al., 2014) according to previous studies (de Tommaso et al., 2011). A trained psychologist explained questionnaires and modalities of response to all participants. We also recorded the Wide Pain Index (WPI) and Symptoms Severity (SS) included in the American College of Rheumatology (ACR) diagnostic criteria (Wolfe et al., 2010) and the Dolor Neuropatique 4 (DN4) for neuropathic pain (Bouhassira et al., 2005). Fatigue was assessed using the Multidimensional Assessment of Fatigue (MAF) (Belza et al., 2018). We evaluated quality of life using the Short-Form 36 (SF-36) Health Survey, and pain severity and interference using the Brief Pain Inventory (Caraceni et al., 1996).

Laser Evoked Potentials (LEP)

Stimulation procedure. Details of the procedure have been previously reported (de Tommaso et al. 2014). Briefly, nociceptive stimulus consisted of laser pulses (wavelength, 10.6 mm; beam diameter, 2 mm; duration of the stimulus 30 ms) generated by a CO_2 laser (Neurolas Electronic Engineering, Florence, Italy). One series of thirty consecutive stimuli were delivered at each stimulation site at an intensity one-step (1.5 W) above the pain threshold, with an interstimulus interval of 10 seconds. Each stimulation series was delivered after an interval of 5 minutes. We stimulated in a random order the right hand-back, the thigh at 20 cm below the anterior iliac spine, and the dorsum of the foot.

Recording procedure. We used a montage with 65 scalp electrodes referred to the nasion, with the ground electrode at Fpz. We considered the Cz derivation for the N2P2 complex and the T3/Fz channel for the N1 component. Two additional electrodes were positioned above the eyebrows, for electro-oculogram (EOG) recording.

LEP analysis. An investigator who was blinded to the clinical condition analyzed LEP recordings of one second, including 100 ms of prestimulus time, at a sampling rate of 256 Hz, using the EEG laboratory tool (version 14.1.1b). In a preliminary analysis, LEP recordings containing transient signals that exceeded 65 µV were automatically deleted. Oculomotor artifacts in any recording channel were removed from the average by ICA algorithm. Other artifacts were visually inspected and affected trial deleted. For each stimulation site, we evaluated the averages of at least 21 valid (artifact-free) responses. LEP were identified based on their latency and distribution; three responses (N1, N2, and P2) were labeled according to

published procedures (Valeriani et al 1996). The N1 component was analyzed at T3 Fz, and the N2 and P2 components were analyzed at the vertex (Treede et al., 2003, Valeriani et al., 1996). The absolute latencies of the scalp potentials were measured at the highest peak of each response component. The amplitude of each wave was measured from the baseline; the peak-to-peak amplitude was taken into consideration for the vertex biphasic LEP component (N2-P2). To estimate habituation phenomenon, the sequence of the first series of responses recorded from hand, thigh and foot sites was divided into three blocks. We considered the averages of at least seven artifact-free consecutive responses for each block (de Tommaso et al 2005). We did not evaluate the N1 habituation, given this wave is small in amplitude and would request more repetitions for reliable averaged responses. The habituation index was defined as the ratio of N2-P2 amplitude between the third and the first group of consecutive responses (3th/1th). A value below 1 expressed a decrement of the response after stimuli repetition and indicated habituation (Rankin CH et al., 2009). We compared amplitude and latency of N1 and N2P2 vertex complex, and habituation index with age-matched normative values from five age groups of at least 10 healthy subjects aged 18 to 72 years for each site of recording (de Tommaso et al., 2014, 2017). Patients were classified as abnormal when values exceeded the 95% confidence interval (CI) ranges. The 95% CI for the habituation index computed in our control series with the same intensity and frequency of stimulation parameters employed in the present study (de Tommaso et al 2014; de Tommaso et al, 2017b) were 0.45-0.61 at the thigh and 0.40-0.65 at the foot. LEPs were recorded only from the right side.

Nerve conduction study (NCS)

NCS was performed according to standard methods of recording and superficial electrodes (Kimura, 2013), using a MICROMED Myoquick 2 channels device (Micromed, Mogliano Veneto, Italy). Right antidromic sensory sural nerve, and motor posterior tibial and peroneal nerve conduction velocity and action potential amplitude were measured (De Vigili et al, 2018) and compared with normative reference values from our laboratory (Table 3).

Skin Biopsy

It was performed using a disposable 3-mm punch at the thigh, 20 cm below the anterior superior iliac spine, and distal leg (10 cm above the lateral malleolus in the territory of the sural nerve), after intradermal injection of 1% xylocaine. Briefly, specimens were fixed (2% paraformaldehyde–lysine–sodium periodate, 4°C overnight), cryoprotected, serially cut with

a cryostat in 50-micron thick sections, and immunostained by polyclonal anti-protein gene product 9.5 (Ultraclone Ltd) using a free-floating standardized protocol. The methodology was fully described in De Vigili et al., 2008; Lauria et al, 2010). IENFD was calculated 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 to sex and age-adjusted normative values (Lauria et al., 2010).

Statistical analysis

The chi square test was applied to establish the association between reduced IENFD density, main comorbidities and LEPs abnormalities. Multivariate ANOVA analysis was used to assess different clinical features (variables) among patients with normal or distally and/or proximally reduced IENFD (IENFD groups as factor), with a post-hoc Bonferroni test. Multivariate linear regression analysis (MANOVA) was also applied to establish the relationships between IENFD and clinical and neurophysiological features.

RESULTS

Patients

Of the 150 patients referred for the first time to our center, 120 were diagnosed with FM, screened for inclusion/exclusion criteria and considered eligible. Eventually, 81 patients agreed to participate in the study (73 women and 7 men; mean age 50 ± 10 years; range 24-75 years). The mean disease duration was 10.69 ± 8.16 years (range 1-20 years). All patients were from Southern Italian regions. Thirty-nine patients suffered from migraine, 39 patients had anxiety and/or depression, 11 patients had previous history of autoimmune disease and/or associated thyroiditis, and 28 patients had more than one comorbidity. All patients used episodically non-steroidal anti-inflammatory drugs, paracetamol or tramadol the intake of whichwas withdrawn at least 24 hours before the examinations.

Skin biopsy

Fifty-nine patients (72.8%) showed reduced IENFD at the thigh, 10 patients (12.3%) had reduced IENFD at both thigh and distal leg, and 12 patients (14.8%) had normal IENFD at both the sites (Table 1). No patient had a small fiber reduction only at the distal site. There was no correlation with comorbidities even when we sub-grouped the patients based on exclusive proximal (P), proximal and distal (PD) and normal (N) IENFD (Table 1S; Table 2S). Patients included in the group with previous history of autoimmune disease had similar

IENFD, as the other patients. In particular, proximal IENFD in patients with previous immune disease was 9.50/mm, whereas it was 9.14/mm in the other patients (ANOVA F p=0.156, n.s; distal IENFD in patients with previous autoimmune disease: 8.22, other patients: 7.66 ANOVA F 0.59, n.s.)

IENFD and clinical features

Considering that the PD group counted only six patients, we did not perform the MANOVA analysis to compare clinical variables among PD, P and N groups. Fatigue, motor and work interference BPI scores negatively correlated with IENFD at the thigh (Fig. 1; Table 2).

Correlation between IENFD and neurophysiological data

NCS was normal in all patients (Table 3). In 23 patients, LEP from hand and thigh had N2P2 amplitude larger than in age-matched control group, which in 10 patients was larger also from the foot. Of them, 18 patients had larger N1 from the hand and thigh, and prolonged P2 from the hand. No significant association with IENFD either at the thigh (chi square 0.74 Fischer 0.76 p=0.49) or distal leg (chi square 0.34 Fischer 0.33 p=0.61) was found (Table 3 S). LEP latencies and amplitudes did not differ between patients with or without reduced IENFD (Table 4 S). Conversely, the habituation index was >0.65 from at least on one site of stimulation in 97.5% of patients (normal values 0.45-0.61 at the thigh and 0.40-0.65 at the foot). Moreover, we found a correlation between habituation index from laser stimulation at the thigh and IENFD at the thigh (Figure 2; Figure 3; Table 4). LEP features did not differ between patients with idiopathic FM and those with previous history of autoimmune disease (Table 5 S).

DISCUSSION

Our study confirmed that most FM patients do not have a small fiber neuropathy but show a variable and mild reduction of IENFD at the thigh, which might lead to the definition of associated proximal or non-length dependent small fiber pathology. The main correlation with LEP recording was the corresponding altered habituation index obtained from stimulation at the thigh. Conversely, we did not find any further correlation with other LEP variables, including amplitude. This may be not surprising also because central amplification can counteract and hinder peripheral nerve fiber loss. Finally, we did not find any correlation with comorbidities or more severe and prolonged disease. The correlation

between reduced IENFD at the thigh and increased fatigue and motor disability scores could have been biased by the relatively larger sample size compared to other IENFD sub-groups.

An atypical pattern of reduced IENFD has been reported in a small cohort of patients (Gemignani et al., 2010) and better characterized in a multicenter retrospective study (Gorson et al., 2008) as non-length dependent small fiber neuropathy. In our study, almost all the patients had normal IENFD at the distal leg, thus confirming that FM features do not include the classical symmetric and distal small fiber neuropathy. Previous studies reported a reduction of IENFD both at proximal and distal sites of lower limbs, with a ratio suggesting a predominant non-length process (de Tommaso et al., 2014a; Germignani et al., 2010; Üçeyler et al., 2013). Our findings confirmed this observation, as in about 70% IENFD was decreased only at the thigh. Only 10 patients had a typical small fiber neuropathy pattern of IENFD decrease, with normal innervation at the thigh. A recent German study on 117 women with FM found the same four patterns of skin denervation, such as normal, distal, proximal, and both distal and proximally (Evdokimov et al, 2019). The nature of such loss of small nerve fibers at proximal sites of the body in FM remains unknown. The hypothesis of a ganglionopathy remains remote, as it presents with distinct clinical and neurophysiological abnormalities (Sghirlanzoni et al, 2005; Gorson et al., 2008, Koike et al., 2008).

Among our included FM patients, no significant association with a history of autoimmune disease was found. Migraine was diagnosed in 48% of FM patients of our cohort, confirming its higher prevalence compared to the rate expected in the general female population (de Tommaso et al., 2009). We did not find any correlation between the pattern of IENFD and anxiety or depression. Similarly, we did not find any correlation with the clinical features as distribution of pain, associated symptoms and FM-linked disability, which were similar in the three groups. A recent study (Evdokimov et al 2019) reported the association between higher disease burden and generalized IENFD reduction. In our study, the small size of subgroups with normal and distal IENFD reduction makes any comparison not reliable. However, the correlation analysis seemed more robust than group analysis in indicating a correlation with fatigue and impairment of motor ability in fibromyalgia patients. Patients with FM have deficits in motor performances, with compromised coordination and control force (Lamoth et al., 2006; Bank et al., 2015; Chang et al, 2018, Gentile et al, 2019). In the last decade, peripheral nerve involvement has been demonstrated in neurodegenerative diseases involving motor circuits as Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS) (Nolano et al, 2008, 2017a; 2017b; Weis et al, 2011; Dalla Bella et al, 2015; Sassone et al 2016) with

partial recovery after motor rehabilitation (Nolano et al, 2018). One report described decrease in fiber nerve density in patients with stroke, pain and central motor symptoms (Cavalier et al 2016). These studies suggested a possible inverse relation between motor activity and small fiber density.

LEPs have not been able to detect a possible small fibers impairment in FM patients. In this and other case series, we observe a wide distribution of FM patients with regard to LEP amplitude in comparison to controls (Van Assche et al., 2020; de Tommaso et al., 2014a; 2017b). The IENF immunostained by the polyclonal anti-protein gene product 9.5 (PGP-9.5) explores several subpopulations of receptors, while LEPs inform merely on the functional status of a small subpopulation of IENF, i.e. the AMH type II nociceptors. Both methods explore different subpopulations of nociceptors, and this would be the reason why we found a lack of correlation between IEFN density and LEP amplitude. Moreover, in previous studies, biopsy-verified decrease of thin fiber density was clearly correlated with LEP amplitude decrease (Rage et al 2010; Casanova-Molla, 2011; Üçeyler et al 2013).

Such discordances between these and the present data may suggest that the decrease of fiber density in FM patients was indeed slight and LEP amplitude also influenced by decreased habituation. In syndromes, like FM, in which an impairment of top-down inhibitory control is likely to occur, and can be intertwined with the degeneration of peripheral nociceptors, reduced habituation could be the expression of central sensitization, as suggested in painful radiculopathies (Hüllemann et al, 2017).

In our study, the most robust neurophysiological finding has been the altered habituation index across repetitive painful laser stimulation at the thigh and its correlation with reduced IENFD at the same site. However, in a situation of mild small fibers denervation, subjects predisposed to central sensitization could have an amplified response at the level of cortical areas comprised in the so-called "salience" network (Legrain et al., 2011). In central sensitization syndromes, deficient habituation to repetitive stimuli is not nociceptive specific, but it involves multimodal stimuli (Choi et al, 2016).The phenomenon of reduced habituation of cortical areas generating LEPs could be facilitated by a decrease of inputs from different nociceptors, partly included in the subpopulations analyzed with the reported methods. Deficient habituation is also described in conditions of basal cortical hypo-activation, confirming that in predisposed subjects, it could be a compensatory phenomenon (Magis et

al, 2013). Patients with more severe IENFD reduction, thus theoretically having more pronounced peripheral afferent loss, showed a progressive potentiation of cortical response to laser-induced nociceptive stimuli, suggesting that central sensitization could drive an amplification of peripheral inputs (Yunus, 2007).

LEP habituation might be more sensitive in capturing this event, which other techniques such as blink reflex failed to demonstrate in other disorders characterized by small fiber impairment like burning mouth syndrome (Kolkka et al, 2019).

A major limit of the present study is the different type of fibers studied with LEPs and skin biopsy. The correlation we found is only indirect, as it only shows that a generic reduction of different peripheral nociceptors corresponds to a pattern of reduced habituation of cortical areas generating LEPs. Methods of analysis focusing on specific nociceptors subtypes, as the C fibers, largely involved in FM pathogenesis (Serra et al, 2014), could better establish a direct relationship between peripheral denervation and central cortical amplification.

General remarks

The main finding of our study is the association between reduced habituation indexes obtained by LEP recording after stimulation of the thigh and reduced IENFD at the same site in FM patients. This result suggests that central sensitization might be the most relevant mechanism underling the disease. Whether the degeneration of peripheral nociceptors can trigger or maintain central sensitization in FM though a positive feedback is unknown.

The mechanism of peripheral nociceptor involvement and the implications in clinical practice remain an unresolved issue. Animal studies suggested that the increase of excitatory tone in a pro-nociceptive brain region might be sufficient to induce the degeneration of small nerve (Desantana et al., 2013). This hypothesis, while suggestive, deserves confirmation in human experiments. The LEP pattern of progressive potentiation in response to weak inputs from peripheral nociceptors, which we described in term of reduced habituation, is in favor of the hypothesis that a mild damage of sensory fibers could contribute to the clinical phenotype of FM in patients predisposed to central sensitization. The search for a common genetic trait combining peripheral nociceptor loss and dysfunction of central nociceptive networks will be a matter of future studies.

Authors' contribution:

E.V: clinical data collection, data analysis, manuscript preparation

K.R. laser evoked potentials recording

G.L: clinical data collection

F.I.: clinical data collections, manuscript editing

G.L.P: study design, manuscript editing

R.L and M.P.: skin analysis

M.D.: psychological evaluation

F.G.: clinical data collection

S.Q: databases management

M.D.T: study design and coordination, data analysis, manuscript preparation

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Legends to the figures

Figure 1

Linear regression analysis between intraepidermal fibers nerve density (IENFD) at the thigh, Brief Pain Inventory (BPI) subscores (motor and work interference) and fatigue in 81 FM patients. Details of the statistical analysis are reported in Table 2.

Figure 2

Linear regression analysis between IENFD at the thigh and N2P2 habituation index after stimulation of the thigh in 81 FM patients. Details of the statistical analysis are reported in Table 4.

Figure 3

Top Time course of the grand average of N2P2 amplitude change across 30 consecutive trials obtained from stimulation at the thigh in 12 patients with normal IENFD (a) and 69 patients

with reduced IENFD at the thigh (b). Negative amplitude is shown in blue, corresponding to the N2 component; positive amplitude is shown in red and corresponds to the P2 wave. Each colored line expresses the amplitude of the grand average of single N2P2 repetitions in the two groups. To note, group b, showed a clear amplification of the P2 component in the last 20 trials, confirmed in the Grand Average reported at the bottom of the figure. The Grand Average of the first series of consecutive LEP responses is represented in blue and third series in green .

		Thigh	
		normal	reduced
		Cases	Cases
Ankle	normal	12	59
		14.8 (3) (Thigh)	8.53 (2.33) (Thigh)
		95% CI:12.8-16.7	95% CI: 9.97-9.14
		10.19 (2.52) (Ankle)	7.77 (2.17) (Ankle)
		95% CI: 8.58-11.79	95% CI:7.22-9.34
	reduced	0	10
			8.22 (2.37) (Thigh)
			95% CI: 6.51-9.91
			4.87 (2.23) (Ankle)
			95% CI: 3.27-6.46

Table 1 FM patients classified for IENFD density (number of single cases exceeding normative values for 2 SD (De Vigili et al, 2008).

Mean, SD and 95% CI of IENFD density in FM patients are reported.

|	R square	Beta (slope)	T	p
duration	0.012	-0.11	-0.84	0.41
Wide Pain Index (WPI)	-0.017	0.02	0.15	0.87
Symptoms Severity (SS)	0.007	-0.083	-0.63	0.52
Fibromyalgia-linked invalidity questionnaire (FIQ)	0.001	0.016	0.118	0.92
Dolor Neuropatique 4 (DN4)	0.001	-0.002	-0.017	0.98
Multidimensional Assessment of Fatigue (MAF)	0.080	-0.28	-2.24	*0.029*
Brief Pain Inventory (BPI) (pain intensity)	0.044	-0.21	-1.81	0.074
Brief Pain Inventory (BPI) (motor interference)	0.065	-0.25	-2.22	*0.029*
Brief Pain Inventory (BPI) (work interference)	0.13	-0.37	-3.35	*0.001*
Short-Form 36 (SF-36) Health Survey (Mental Health Index)	0.025	0.15	1.4	0.16
Short-Form 36 (SF-36) Health Survey (Physical Health Index)	0.04	0.2	1.79	0.077

Table 2 Linear regression analysis between main clinical features and proximal IENFD density in 81 FM patients.

	Sural SNAP Amplitude (µV)	Sural NCV (m/s)	Peroneal CMAP Amplitude (mV)	Peroneal NCV (m/s)	Tibial CMAP Amplitude (mV)	Tibial NCV (m/s)
Mean	11.27	43.44	7.21	50.24	15.92	43.24
SD	7.10	18.98	2.46	4.32	8.38	17.03
Normal values	>6	>46	>3	>43	>5	>41

Table 3. Mean and Standard Deviations (SD) of nerve conduction values in 81 FM patients. No single case exceeded the normal values of the laboratory.

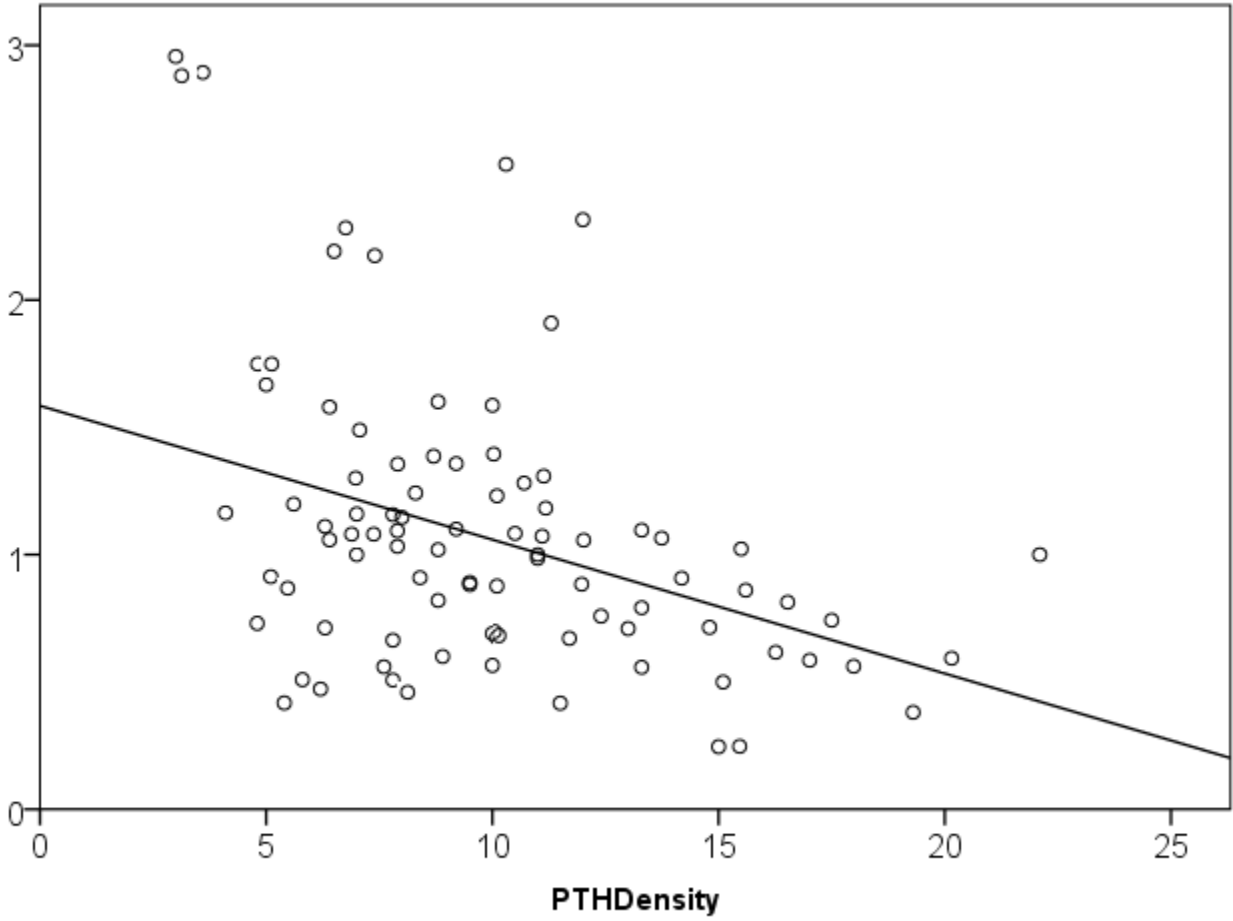|	R square	beta	t	p
N2P2 habituation index	0.102	-0.319	-2.60	*0.012*
N2P2 amplitude	0.0001	0.015	0.12	0.94

Table 4 Linear regression analysis between LEP amplitude and habituation index obtained by the thigh stimulation   and proximal IENFD density in 81 FM patients.
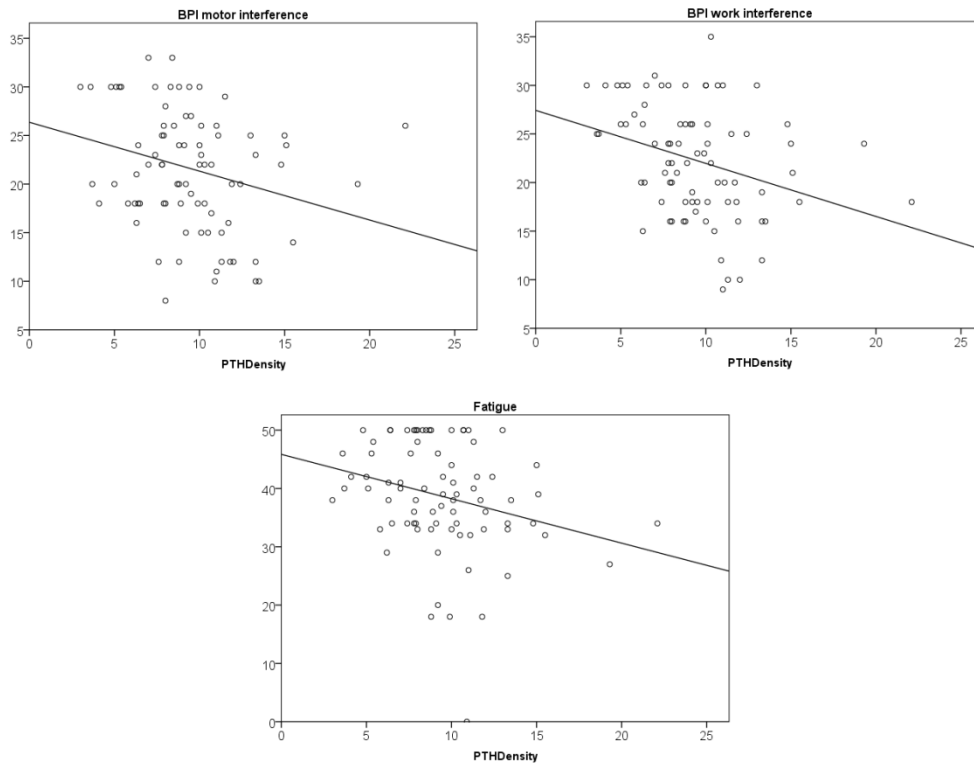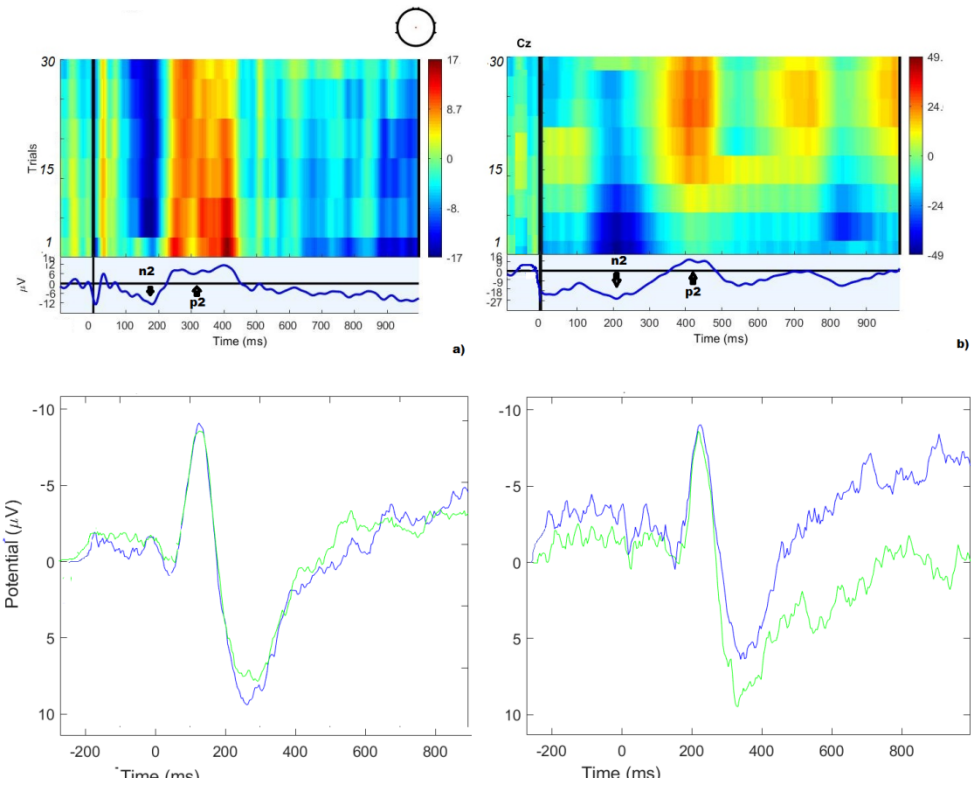
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