Received: 26 April 2019 | Revised: 28 October 2019 | Accepted: 29 October 2019

DOI: 10.1002/ejp.1501

ORIGINAL ARTICLE

EJP European Journal of Pain

Fibromyalgia syndrome—A laser-evoked potentials study unsupportive of small nerve fibre involvement

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Funding information
FRS-FNRS Belgium

Abstract

Background: Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterized by widespread pain and a variety of non-pain symptoms. Central sensitivity phenomena are found consistently in FMS. Additionally, several researchers proclaimed that a subgroup of FMS patients may present with unrecognized peripheral small fibre neuropathy (SFN). Laser-evoked brain potentials (LEP) are considered as a reliable method for the functional assessment of the thermo-nociceptive system, including the evaluation of SFN.

Objectives: The aim of this retrospective study was to estimate the prevalence of thermo-nociceptive system dysfunction based on LEPs in FMS.

Methods: LEP recordings of 92 FMS patients and 39 age and gender-matched healthy controls were selected from a database collected between 2003 and 2012 with standardized settings for laser stimulation and EEG recording. The N1, N2 and P2 LEP components were identified and characterized by peak latency and amplitude.

Results: None of the FMS patients showed signs of loss of function of the nociceptive responses evoked by A δ-nociceptor activation, compared to healthy controls. 6.5% of the FMS patients had N2-P2 peak-to-peak amplitudes above the upper limit of the 99%-confidence interval. N2-P2 peak-to-peak amplitudes were negatively correlated with age, without age-related differences between groups.

Conclusions: The characteristic signs of a damaged thermo-nociceptive system as revealed by LEPs were absent in this large cohort of FMS patients.

Significance: The present research does not support the hypothesis that small fibre neuropathy is a significant contributor to the pathophysiology of FMS.

1 | INTRODUCTION

Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterized by widespread pain, fatigue and sleep disorders (Arnold et al., 2018). The prevalence of FMS in the general population is estimated between 1.1% and 6.4% (Vincent et al., 2013).

The pathophysiology of FMS is subject to a vast literature and generates vigorous debates. The clinical phenotypes of FMS patients are heterogeneous and many pathological findings have been observed in different somatic systems. Patients may present with endocrine, cardiovascular, gastrointestinal and neurological disturbances being triggered by internal or external events in a context of genetic susceptibility (for review: Arnold et al., 2018). Central sensitization phenomena are found consistently in these patients, as often in the context of chronic pain syndromes (Woolf, 2010; Yunus, 2007). The pathophysiology of FMS for a long time was considered

to be the complex product of various mechanisms including operant learning mechanisms, hyporeactivity of the hypothalamo-adrenal axis, neurotransmitter disturbances and an abnormal balance between pro- and anti-inflammatory cytokines (Sommer et al., 2008, 2012; Uçeyler, Häuser, & Sommer, 2011; Uçeyler et al., 2006). Such notion of complexity seems to have abated following the description of abnormal skin biopsies in FMS patients in 2013. A number of researchers proclaimed that a subgroup of FMS patients may present with unrecognized peripheral small fibre neuropathy (SFN) or pathology (Giannoccaro, Donadio, Incensi, Avoni, & Liguori, 2013; Oaklander, Herzog, Downs, & Klein, 2013; Uçeyler et al., 2013; de Tommaso et al., 2014); some considering that SFN may be a key feature of the pathophysiological mechanisms leading to chronic widespread pain in FMS (Doppler, Rittner, Deckart, & Sommer, 2015; Serra et al., 2014). This has led to recommending the routine use of skin punch biopsy to diagnose SFN in patients with FMS (Levine & Saperstein, 2015). Other authors acknowledge the presence of decreased intra-epidermal nerve fibre (IENF) density upon skin punch biopsy in FMS as a non-specific structural finding (Arnold et al., 2018; Clauw, 2015). It is not clear at present if SFN should be considered as part of the disease process, as an incidental concomitant disease, as a non-specific finding or as an alternative diagnosis for FMS. It may be useful to investigate the presence of small fibre pathology in the FMS population by using alternative functional investigations and adapted statistical methods to overcome test biases.

Laser-evoked brain potentials (LEP) are considered as a reliable method for the functional assessment of the thermo-nociceptive system, including the evaluation of small fibre neuropathies (Cruccu et al., 2008; Valeriani, Pazzaglia, Cruccu, & Truini, 2012). Late vertex potentials are elicited by the selective activation of slow-conducting Aδ- and C-fibre nociceptors sensitive to non-invasive phasic thermal stimulation (Bromm, Frieling, & Lankers, 1991; Spiegel, Hansen, Baumgartner, Hopf, & Treede, 2003; Treede, Lorenz, & Baumgärtner, 2003). The diagnostic performance of LEP to diagnose SFN has been ascertained, specifically in diabetic neuropathy (Casanova-Molla, Grau-Junyent, Morales, & Valls-Solé, 2011; Di Stefano et al., 2017; Ragé et al., 2011). If a subgroup of FMS patients has functional disturbances of peripheral small nerve fibres related to SFN, we expect these to be detectable with LEP testing. Two papers reported low/absent LEP amplitudes in subgroups of FMS patients suggesting SFN (de Tommaso et al., 2014, 2017). Several early LEP studies in FMS described higher signal amplitudes compared to healthy controls, considered to mirror hypervigilance or attentional mechanisms (de Tommaso et al., 2017; Garcia-Larrea et al., 2002; Gibson, Littlejohn, Gorman, Helme, & Granges, 1994; Lorenz, Grasedyck, & Bromm, 1996). None of these previous papers assessed the diagnostic accuracy of LEPs for diagnosing FMS.

The aims of the present retrospective study were to estimate the prevalence of thermo-nociceptive system dysfunction and assess the diagnostic accuracy of LEPs in a large sample of FMS patients.

2 | METHODS

2.1 | Data collection

Laser-evoked potentials recordings were retrieved retrospectively from a database of patient files of the Department of Physical Medicine and Rehabilitation of the Cliniques universitaires Saint-Luc (Brussels, Belgium). All LEP recordings acquired between January 2003 and December 2012 were screened for inclusion. During this time period, LEP recordings were carried out by the same experienced examiner (LP) using a standardized examination protocol. LEP recordings were classified according to the site of laser stimulation (hand or other site) and according to the clinical diagnosis at the time of referral for further electrophysiological exploration of the nociceptive system. For each patient, the original medical file was retrieved in order to collect demographic and clinical data of patients and to confirm the clinical diagnosis at the time of testing. The diagnosis of fibromyalgia syndrome was based on the criteria of the American College of Rheumatology (Wolfe et al., 1990), as confirmed by the referring physician. In case of doubt regarding the clinical diagnosis, referring physicians (mainly rheumatologists and physiatrists working at different hospitals in the network of the Université catholique de Louvain (UCLouvain), Belgium) were contacted by phone and by mail to obtain all needed information. Other exclusion criteria were: age under 18 years old, several LEP examinations in the same patient (only the first examination was included), bad signal to noise ratio, documented use of benzodiazepines within 24 hr prior to LEP recording, any central or peripheral nervous system disorder existing prior to or at the time of LEP recording. This data collection procedure allowed to ensure that selected LEP recordings pertained to FMS patients without any other known co-morbidity.

During the same time period (2003–2012) laser evoked potentials with the same standardized settings and supervised by LP, were acquired from healthy volunteers in the context of two previously published clinical trials (Hatem et al., 2010; Ragé et al., 2011). From a chronological perspective, the LEP acquisitions of these healthy controls were intermingled with those of FMS patients.

This study was approved by the Ethics Committee of Brugmann University Hospital (Brussels, Belgium, Ref: CE2013/103) and by the Ethics Committee of Cliniques universitaires Saint-Luc—UCLouvain (Brussels, Belgium, Ref: CE2014/014).

2.2 | Laser-evoked potentials

Laser-evoked potentials were acquired during a strictly standardized stimulation and recording procedure supervised by the same investigator (LP). Before starting LEP acquisitions, absolute detection threshold (C-fibre related), defined as the lowest stimulus intensity detected with a probability of 0.5, and pinprick detection threshold (Aδ-nociceptor related) were determined using the method of limits. Laser stimuli were applied to the dorsal surface of the hand. Aδ-nociceptor and C-nociceptor activation were inferred from the reaction times of the subjects (<650 ms for Aδ-nociceptor; otherwise for C-nociceptors). Reaction times (RTs) were measured by instructing the subject to press a micro-switch mounted on a hand-controller as soon as any type of sensation at the stimulation site was perceived. The trade-off between RTs ascribed to Aδ-nociceptor activations and those in response to C-nociceptor activations after CO_2-laser heat stimuli was set at 650 ms according to previous studies (Hatem et al., 2010; Ragé et al., 2011). The laser fluence for LEP recordings was determined in such a way that energy density at target was supraliminal for Aδ-nociceptor activation (9.4 ± 1.1 mJ/mm^2) and evoked a clear pricking and burning sensation in all subjects. Subjects were exposed to three consecutive series of 10 suprathreshold (for type II AMH nociceptors) laser stimuli per stimulation site (30 laser stimuli in total), delivered by a CO_2 laser (stimulus duration: 50 ms; beam diameter: 10 mm) at the dorsal side of the hand. Each laser stimulus was followed by a variable interstimulus interval of 5–10 s. The target spot was repositioned slightly after each stimulation, pseudo-randomly, to avoid skin damage by overheating, to minimize habituation and to fence off nociceptor sensitization or fatigue.

Nineteen Ag-AgCl cutaneous scalp surface electrodes were positioned according to the International 10–20 System of EEG electrodes, referenced to the earlobes. In addition, an electro-oculogram of the right eye was recorded with two disposable Ag-AgCl surface electrodes, to monitor ocular movement and eye blink artefacts. The ground electrode was placed at the unstimulated fore-arm. EEG signals were sampled at 167Hz, amplified and stored on a hard disk for off-line processing using BrainVision Analyzer 1.05 (Brain Products GmbH, Germany). The continuous EEG recording was segmented into 3,000 ms long epochs ranging from −500 to 2,500 ms relative to stimulus onset (512 data points). Technical and blink artefacts (EOG-contaminated sweeps) were rejected after visual inspection. A band pass filter of 0.1–30 Hz (80 dB/decade) was applied, followed by a baseline correction, based on the −500 to 0 ms pre-stimulus interval, and by time-averaging of the epochs (Hatem et al., 2012). The N2 and P2 peaks were determined at the vertex electrode Cz, for each subject in each averaged waveform, by visually identification. N2 latencies, P2 latencies and N2-P2 peak-to-peak amplitudes were used for analyses. Because of its difficulty to be identified by visual inspection, the N1 peak was assessed with an automated single-trial analysis as described previously (Hatem et al., 2012), at the contralateral temporal electrode (T3 or T4) referenced to Fz. N1 latencies and peak-to-baseline amplitudes were used for analyses.

2.3 | Statistical analysis

Normality of data distributions was assessed with the Shapiro–Wilk's normality test and a log-transformation was applied when indicated. Chi-square tests were used to assess differences between the frequencies of categorical variables. Further differences between groups were analysed by using the two-sided Student's *t* test, ANOVA or the non-parametric Mann–Whitney *U* test, as appropriate. An ANCOVA with factors AGE and GROUP, was used for comparing LEP amplitudes between groups given the well-known strong negative correlation of LEP peak amplitude with age (Truini et al., 2005). The diagnostic performance of LEPs was estimated by Receiver Operating Characteristic (ROC) analysis based on classification with binomial logistic regression using Matlab (The Mathworks). Subjects' N2-P2 amplitudes were classified as normal/abnormal by logistic regression with a decision boundary based on the optimal operating point of the ROC curve. Furthermore, the number of FMs patients with abnormal individual responses, i.e. deviating more or less than 2.5 standard deviations from the average of healthy controls, was calculated with and without correction for age.

In all cases, a *p*-value below .050 was considered as significant. The software package SPSS (version 17.0) was used for statistical analyses.

3 | RESULTS

The LEP recordings of 92 out-clinic FMS patients met all requirements and were included in this study (Figure 1), as well as the LEP recordings of 39 healthy controls (HC). Table 1 shows the demographic characteristics of both groups. No significant differences were observed between groups with regards to gender ratio or age. Of note, age had a Gaussian distribution and similar parameters in both groups (Table 1).

Laser-evoked potentials exhibited a clear biphasic wave, identified as the N2-P2 complex, maximum at the vertex in all cases. The N1 peak was identified in all healthy controls and in 92% of FMS patients (85 of 92 FMS patients). The electrophysiological parameters of N1, N2 and P2 peaks, as well as the absolute detection (C-nociceptor related) and pinprick detection (Aδ-nociceptor related) thresholds are reported in Table 1. The detection thresholds elicited

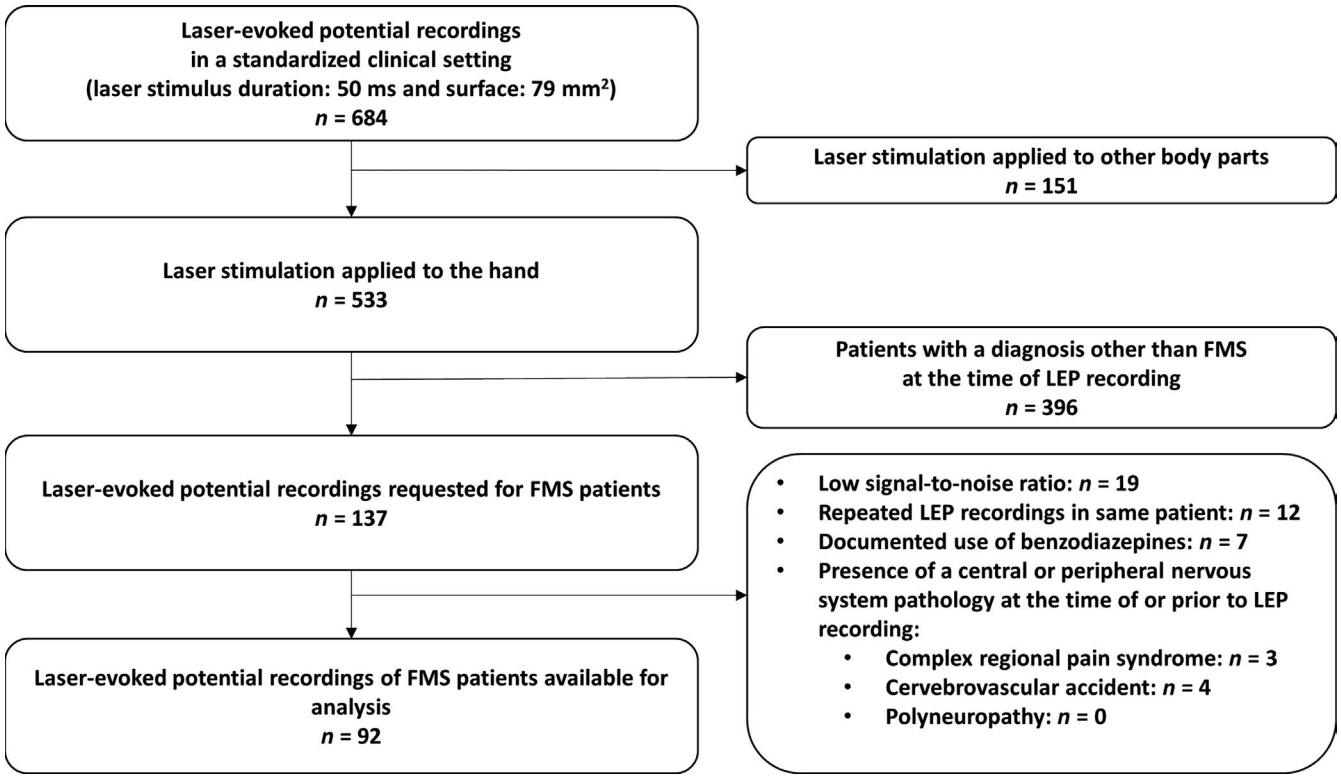

FIGURE 1 Data collection of laser-evoked potential recordings in patients with fibromyalgia syndrome

TABLE 1 Demographic features and laser-evoked potential parameters (mean ± *SD*) after laser stimulation of the hand dorsum in fibromyalgia patients (FMS) and healthy controls (HC)

	FMS	HC	Statistics	*p*-value
n	92	39		
Gender (M/F)	29/63	14/25	$\chi^2 = 0.081$	>.500
Age				
Mean ± *SD* (yrs)	49 ± 11.3	45 ± 12.6	$t = 1.620$	.108
Range (yrs)	21–84	20–71		
LEP (median and interquartile range)				
N1 latency (ms)	201 [31]	202 [44]	$z = -0.599$	>.500
N2 latency (ms)	243 [41]	246 [26]	$z = -0.038$	>.500
P2 latency (ms)	359 [74]	377 [67]	$z = -1.039$	.299
N1 amplitude (μV)	−6.5 [−8.2]	−4.8 [−5.8]	$z = -1.477$	.140
N2-P2 amplitude (μV)	26.6 [24.6]	29.6 [22.7]	$z = -0.108$	>.500
Ln[N2-P2]	3.28 [0.90]	3.39 [0.81]	$t = 0.341$	>.500
Thresholds elicited by laser stimulation (Mean ± *SD*, mJ/mm^2)				
Absolute detection threshold (C-nociceptor related)	3.4 ± 1.67	3.4 ± 0.80	$F = 0.0001$	>.500
Pinprick detection threshold (Aδ-nociceptor related)	6.5 ± 2.11	6.3 ± 0.85	$F = 0.2609$	>.500

by laser stimulation were not significantly different between both groups (ANOVA: absolute detection threshold: $F = 0.0001$, $p > .500$; pinprick detection threshold: $F = 0.2609$, $p > .500$).

As expected, the distributions of the N1, N2 and P2 latencies were positively skewed. The N1 latencies and amplitudes were not significantly different between groups (Mann–Whitney: N1 latency: $z = -0.599$, $p > .500$; N1 amplitude:

$z = -1.477$, $p = .140$). N2 and P2 latencies were not significantly different between groups (Mann–Whitney: N2 latency: $z = -0.038$, $p > .500$; P2 latency: $z = -1.039$, $p = .299$).

In the FMS-group, the distribution of N2-P2 amplitudes was slightly positively skewed (skewness = +1.097) and not normally distributed (W = 0.907, $p < .0001$). In the HC-group, N2-P2 amplitudes were normally distributed (skewness = +0.414; W = 0.971, $p = .391$). We applied a logarithmic transformation on the N2-P2 amplitudes to achieve normal distributions in both groups. N2-P2 amplitudes were not significantly different between groups (Student t: 0.341, $p > .500$) (Table 1). A supplementary analysis was performed using AGE as a covariate. Age previously was shown to correlate strongly and negatively with the N2-P2 peak amplitudes of LEP (Truini et al., 2005). In the present study, the range of ages in both groups was large, extending across six decades. ANCOVA showed a decrease in LEP amplitude with increasing age (ANCOVA factor AGE: $F = 33.55$; $p < .001$), but it occurred in a similar fashion for both groups (ANCOVA factor GROUP: $F = 1.52$; $p = .218$) (Figure 2).

Finally, the absence of a subgroup of patients with either increase or decrease in LEP N2-P2 amplitude as compared to the HC group was also confirmed by a binomial classification procedure using logistic regression. The N2-P2 LEP amplitude was unable to distinguish subjects with our without the condition of FMS since the area under the ROC curve (AUC) was 0.521 [0.394–0.632]. The odds ratio of 1.17 indicates that the test gives similar results for the FMS group as for the HC group. Further analysis showed that three patients (or 3.3%) presented with a N2-P2 amplitude larger than 2.5 standard deviations above the average of healthy controls. After correction for age, that number increased to six patients (or 6.5%) with a mean age of 42 years (range 30–53 years). Such very large LEP amplitudes were not observed in healthy controls. None of the patients had N2-P2 amplitudes smaller than 2.5 *SD* below the average of healthy controls. The implications of these results will be discussed below.

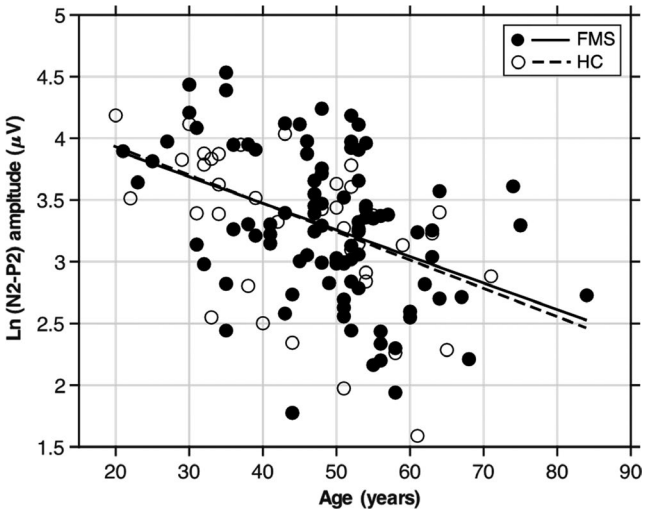

FIGURE 2 Scatter plot with the logarithm transformed N2-P2 amplitudes (μV) of the laser-evoked potential recordings as a function of age (years) in fibromyalgia patients (FMS - black dots) and in healthy controls (HC open dots) after hand dorsum stimulation. The solid line is the least-squares regression line for the FMS (Y = 4.34–0.022X; $R^2 = .158$; $p < .001$). The dashed line is the least-squares regression line for the HC group (Y = 4.39–0.024X; $R^2 = .279$; $p < .001$). ANCOVA finds a decrease in LEP amplitude with increasing age (ANCOVA factor AGE: $F = 33.55$; $p < .001$), occurring in a similar fashion for both groups (ANCOVA factor GROUP: $F = 1.52$; $p = .218$)

4 | DISCUSSION

In this retrospective study of a large sample of patients with fibromyalgia syndrome, laser-evoked potentials showed no signs of loss of function of the nociceptive responses evoked by Aδ-nociceptor activation, compared to a gender- and age-matched group of healthy controls. A small subgroup (6.5%) of the FMS patients had N2-P2 peak-to-peak amplitudes above the upper limit of the 99%-confidence interval. N2-P2 peak-to-peak amplitudes were negatively correlated with age, without age-related differences between groups.

To our knowledge, this is the first study to report consistently normal LEPs in a large group of FMS patients with only a fraction of patients presenting with abnormally large LEPs and no patients presenting with abnormally small LEPs. These results are remarkable as previous research groups have shown distinct LEP patterns in FMS patients compared to other groups of patients or to controls (de Tommaso et al., 2014, 2017; Garcia-Larrea et al., 2002; Gibson et al., 1994; Lorenz et al., 1996).

A small subgroup of patients (6.5% of the study sample after correction of LEPs for age) had LEPs with N2-P2 amplitudes lying above the upper limit of the 99% confidence interval. These high amplitude signals also have been described in other LEP studies, comparing patients with FMS to age-matched healthy controls (de Tommaso et al., 2017; Gibson et al., 1994; Lorenz et al., 1996) or to patients with central neuropathic pain (Garcia-Larrea et al., 2002). Enhanced LEPs are considered to mirror hypervigilance or attentional mechanisms, in the presence of undamaged nociceptive pathways (de Tommaso et al., 2017; Garcia-Larrea et al., 2002; Lorenz et al., 1996).

With regards to the absence of pathologically small LEPs, the present study raises several questions regarding the diagnostic accuracy of methods to assess SFN, the statistical methods used to describe disease prevalence, the biases linked with the heterogeneity of FMS clinical phenotypes and the technical characteristics of laser stimulation.

Small fibre neuropathy is essentially a clinical diagnosis. Diagnostic criteria have been proposed based on the combination of abnormal neurological examination and both

abnormal quantitative sudomotor axon reflex (sweat) test and quantitative sensory testing (QST) (Blackmore & Siddiqi, 2017). Definite diagnosis of diabetic SFN needs (1) the presence of length-dependent neuropathic symptoms, (2) normal nerve conduction studies and (3) abnormal QST or abnormal IENF density in skin punch biopsies (Tesfaye et al., 2010). Due to the lack of gold standard for diagnosing SFN, the diagnostic value of each diagnostic method is assessed against other available techniques. The prevalence of abnormal findings on a diagnostic test is biased by the characteristics of the test and by the prevalence of disease in the studied population. For rare diseases (as is SFN in the general population with a prevalence of 0.053% (Peters et al., 2013)), even a test that seems very accurate, does not necessarily produce a relevant group of true-positives (Rogan & Gladen, 1978). Given that SFN prevalence is very low in the general population, we may ask ourselves if diagnostic tools (such as skin punch biopsy) that detect structural abnormalities could overestimate the presence of SFN. In function of the cut-off that is used, the sensitivity of skin punch biopsy is rather low (sensitivity: 35% and specificity: 95% when the 5th percentile is considered as the cut-off), or medium (sensitivity: 78% and specificity: 64% when the ROC-analysis optimized cut-off is used) (Lauria et al., 2010). In a retrospective study, the sensitivity and specificity of skin punch biopsy of the distal leg for diagnosing SFN (due to a variety of etiologies) were 82.8% and 90%, respectively (optimized cut-off based on ROC curve analysis). The standard against which the diagnostic value of skin punch biopsy was computed included a combination of clinical criteria, QST and skin punch biopsy, and not a distinct gold standard (Devigili et al., 2008). A recent meta-analysis and systematic review reported that the pooled prevalence of SFN in fibromyalgia is 49% (95%CI: 38%–60%) (Grayston et al., 2018). All studies included in this meta-analysis established the diagnosis of SFN on structural findings: the reduction in IENF density upon skin punch biopsy or corneal confocal microscopy. From a statistical point of view, it is disturbing to infer the prevalence of SFN in a patient population from the results of one test; especially as this structural test does not qualify by itself for having the disease (see above: combination of diagnostic criteria for SFN). A major limitation of the meta-analysis was recruitment bias, since in six of eight studies, patients were enlisted by a Department of Neurology and had enough concern over the possibility of having SFN that they accepted the testing. In conclusion, reasonable doubt may be raised over the high prevalence of SFN (or small fibre pathology) previously described in the FMS population by using structural findings such as IENF density, for common statistical reasons (lack of gold standard, low prevalence in the general population, use of optimized cut-off based on ROC analysis).

We will now discuss the relevance of using LEPs for detecting abnormal functioning of small nerve fibres. Changes in LEP responses do not allow for a direct anatomical/structural characterization of the underlying disease. In the absence of central nervous system disease, a diminished LEP amplitude is interpreted as due to the functional loss of peripheral nociceptors. Hence, laser-evoked potentials have been used successfully to detect SFN. For instance, Casanova-Molla et al. (2011) calculated the diagnostic efficiency of LEPs in reflecting the loss of IENF using skin punch biopsies. ROC analysis of LEP amplitudes confirmed the high performance of LEPs with an AUC of 0.85, a sensitivity of 78.2% and a specificity of 86.1%, yielding an odds ratio of 22.2. Di Stefano et al. (2017) have described the diagnostic accuracy of LEP in patients with symptomatic diabetic SFN compared to skin punch biopsy as a gold standard. The sensitivity and specificity were, respectively, 78% and 81% (giving an odds ratio of 15.1) based on age-corrected normative values of the N2-P2 complex amplitude when using a cut-off at mean—2SD. When the cut-off value was optimized based on the ROC analysis, sensitivity and specificity of LEPs were 78% and 96%, respectively. Comparable results were obtained by Ragé et al. (2011) in asymptomatic diabetic neuropathy, provided they used a similar small laser beam of 5 mm diameter. With a laser stimulation protocol strictly identical to the one used in the present study (e.g., a laser beam diameter of 10 mm), the diagnostic performance of LEP amplitude was less but still reasonably good with an AUC of 0.735 ± 0.168, a sensitivity of 74% and a specificity of 64% yielding an odds ratio of 5.1. Of note, sensitivity and specificity are two test characteristics that are constant across different populations with different prevalence of disease (Rogan & Gladen, 1978). Thus, LEPs appear as a reliable clinical diagnostic tool for SFN, albeit not invasive like skin punch biopsy and easily repeatable for follow-up. Consequently, the diagnostic performance of LEPs should have been sufficient to detect patients with SFN in the present FMS population. The absence of patients with reduced LEP amplitude indicative of SFN in our FMS cohort cannot be explained by characteristics of the test or by disease prevalence in the sample.

The major limitation of the present study is the retrospective retrieval of clinical data. Though the stimulation protocol and the electrophysiological data gathering were rigorous over time, it is difficult to exclude biases linked to the referral of patients for electrophysiological assessment (e.g., screening for SFN by other methods preceding the referral, use of ACR 1990 clinical classification criteria for FMS).

The absence of LEP abnormalities in the larger part of the studied FMS patients' cohort also has to be interpreted in function of the laser stimulation protocol evoking primarily Aδ-nociceptor related responses activation. It could be argued that the SFN observed in FMS in other studies may be linked predominantly to unmyelinated C-fibres pathology, and not to A-nociceptors. Granot et al. (2001) showed evidence of local peripheral sensitization of C-fibre function

at upper limb tender points of FMS patients. In contrast, de Tommaso et al. (2014, 2017) showed clear abnormal Aδ-nociceptor related LEP N2-P2 complexes, without ultra-late C-nociceptor related responses. In the present data set, none of the FMS patients or healthy controls showed ultra-late C-fibre related brain responses upon Aδ-nociceptor laser stimulation. Also, the absolute detection thresholds (C-nociceptor related) elicited by laser stimulation were similar in FMS patients and in healthy controls. However, these threshold data should only be considered as an indirect and weak argument. In the clinical context of this study, the thresholds merely were measured to ensure that laser stimulation would be above the Aδ-nociceptor activation threshold. The method of limits for finding thresholds, even with trade-offs based on reaction times, should not be considered as robust enough for interpretation (Jankovski, Plaghki, & Mouraux, 2013). Consequently, our dataset cannot conclude on the presence or not of C-fibre dysfunction. Several specific techniques exist for the selective assessment of C-fibre afferent function with evoked potentials (Jankovski et al., 2013; for a review see: Madsen, Finnerup, and Baumgärtner (2014). A dissociation between Aδ and C-fibre function has been reported scarcely in clinical situations. Lankers, Frieling, Kunze, and Bromm (1991) observed ultra-late LEPs in a patient with hereditary motor and sensory neuropathy Type 1 affecting myelinated fibres with selective preservation of C-fibres. Our research group also has observed occasionally ultra-late LEPs (Caty, Hu, Legrain, Plaghki, & Mouraux, 2013) using an Aδ-nociceptor laser stimulation protocol identical to the one used in the present study. To our knowledge, the reverse situation, i.e. dysfunctional C-fibre afferents in the presence of fully preserved Aδ-fibre function, has not been investigated systematically with laser stimulation in clinical investigations of sensory neuropathies and no information is available on this matter. Thus, C-fibre stimulation paradigms should be more systematically performed in future research and particularly in clinical settings.

The stimulation parameters used in our standardized setting could be inadequate to disclose abnormal nociceptor habituation to repeated painful stimuli in FMS: we applied laser stimuli with an inter-stimulus interval (ISI) randomly varying from 5 to 10 s. Based on the stimulation paradigms and results obtained by other research groups, it could be hypothesized that longer ISI intervals (≥10 s) are more effective at diminishing nociceptor habituation (de Tommaso et al. (2011, 2017): fixed 10-s ISI; Lorenz et al. (1996): random 10-s to 15-s ISI; Garcia-Larrea et al. (2002): 10 ± 2 s ISI; Granot et al. (2001) fixed 5-s ISI).

Finally, only the LEP recordings after hand laser stimulation were included. This seemed reasonable as previous research has shown that in FMS patients the LEP peak amplitudes after hand stimulation do not differ significantly from those after lower limb stimulation (de Tommaso et al., 2011).

5 | CONCLUSIONS

The characteristic signs of a damaged thermo-nociceptive system as revealed by LEPs were absent in this retrospective study of a large cohort of patients with fibromyalgia syndrome. The previously reported high prevalence of SFN in subgroups of FMS patients may have been biased by the (lack of) clinical reliability of diagnosis, the diagnostic accuracy of techniques and the choice of statistical analyses used to calculate prevalence. The present results do not support the hypothesis that SFN is a significant contributor to the pathophysiology of FMS.

ACKNOWLEDGEMENTS

We thank Michael Ragé (MD, PhD) for his contribution in collecting clinical and electrophysiological data.

AUTHOR CONTRIBUTIONS

LP and SH designed the study protocol and collected the electrophysiological data. DVA, LP, EM and SH collected the clinical data. DVA encoded the data. LP, SH and DVA performed the data analysis and statistical analysis. SH, DVA and LP wrote the manuscript. All authors discussed the results and commented on the manuscript.

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How to cite this article: 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*. 2019;00:1–9. https://doi.org/10.1002/ejp.1501