Springer

Dear Author,

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For fax submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/ corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
  Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL: http://dx.doi.org/[DOI].
If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information go to: http://www.link.springer.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

ArticleTitle	Update on laser-evoked potential findings in fibromyalgia patients in light of clinical and skin biopsy features	
Article Sub-Title		
Article CopyRight	Springer-Verlag Berlin Heidelberg (This will be the copyright line in the final PDF)	
Journal Name	Journal of Neurology	
Corresponding Author	Family Name	**Tommaso**
	Particle	**de**
	Given Name	**Marina**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	m.detommaso@neurol.uniba.it
Author	Family Name	**Nolano**
	Particle	
	Given Name	**Maria**
	Suffix	
	Division	Neurology Department
	Organization	“Salvatore Maugeri” Foundation IRCCS-Medical Center of Telese
	Address	Benevento, Italy
	Email	
Author	Family Name	**Iannone**
	Particle	
	Given Name	**Florenzo**
	Suffix	
	Division	Rheumatology Unit, Medical School
	Organization	University of Bari Aldo Moro
	Address	Bari, Italy
	Email	
Author	Family Name	**Vecchio**
	Particle	
	Given Name	**Eleonora**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Ricci**
	Particle	
	Given Name	**Katia**

	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Lorenzo**
	Particle	
	Given Name	**Marta**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Delussi**
	Particle	
	Given Name	**Marianna**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Girolamo**
	Particle	
	Given Name	**Francesco**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Lavolpe**
	Particle	
	Given Name	**Vito**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	
Author	Family Name	**Provitera**
	Particle	
	Given Name	**Vincenzo**
	Suffix	
	Division	Neurology Department

	Organization	“Salvatore Maugeri” Foundation IRCCS-Medical Center of Telese
	Address	Benevento, Italy
	Email	
Author	Family Name	**Stancanelli**
	Particle	
	Given Name	**Annamaria**
	Suffix	
	Division	Neurology Department
	Organization	“Salvatore Maugeri” Foundation IRCCS-Medical Center of Telese
	Address	Benevento, Italy
	Email	
Author	Family Name	**Lapadula**
	Particle	
	Given Name	**Giovanni**
	Suffix	
	Division	Rheumatology Unit, Medical School
	Organization	University of Bari Aldo Moro
	Address	Bari, Italy
	Email	
Author	Family Name	**Livrea**
	Particle	
	Given Name	**Paolo**
	Suffix	
	Division	Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital
	Organization	Bari Aldo Moro University
	Address	Via Amendola 207 A, Bari, 70124, Italy
	Email	

Schedule	Received	29 September 2013
	Revised	23 November 2013
	Accepted	4 December 2013

Abstract	In fibromyalgia (FM), reduced habituation of laser-evoked potentials (LEPs) suggests a dysfunction of pain processing at a central level. In this study, we aimed to further examine the nociceptive pathways at the peripheral to the central level in a large group of FM patients by means of LEPs and skin biopsy, in light of healthy controls findings and main clinical features. One hundred and ninety-nine FM patients and 109 age- and sex-matched controls were submitted to LEPs by the dorsum of the right hand and the skin over the right chest and knee tender point stimulation. Skin biopsy was performed in 21 randomly selected FM patients and 60 age- and sex-matched controls. The mean N2–P2 amplitude was reduced in the whole FM group, with normal or even increased values in patients with migraine as comorbidity and reduced values in other patients including those presenting with distal sensory deficits. All patients had reduced N2–P2 habituation in respect to controls. In the FM group, LEPs habituation was correlated with pain at tender points and bad quality of life. Epidermal fiber density was significantly reduced in FM patients versus controls, and correlated with N2–P2 amplitude by the hand and chest tender-point stimulation. Dysfunction in the nociceptive system at both the central and peripheral levels may concur to explain phenotypical eterogeneity and clinical symptom complexity in fibromyalgia.

Keywords (separated by '-')	Fibromyalgia - Laser-evoked potentials - Skin biopsy - Peripheral and central nervous system dysfunction
Footnote Information	

J Neurol
DOI 10.1007/s00415-013-7211-9

ORIGINAL COMMUNICATION

Update on laser-evoked potential findings in fibromyalgia patients in light of clinical and skin biopsy features

Marina de Tommaso · Maria Nolano · Florenzo Iannone · Eleonora Vecchio · Katia Ricci · Marta Lorenzo · Marianna Delussi · Francesco Girolamo · Vito Lavolpe · Vincenzo Provitera · Annamaria Stancanelli · Giovanni Lapadula · Paolo Livrea

Received: 29 September 2013 / Revised: 23 November 2013 / Accepted: 4 December 2013

Abstract In fibromyalgia (FM), reduced habituation of laser-evoked potentials (LEPs) suggests a dysfunction of pain processing at a central level. In this study, we aimed to further examine the nociceptive pathways at the peripheral to the central level in a large group of FM patients by means of LEPs and skin biopsy, in light of healthy controls findings and main clinical features. One hundred and ninety-nine FM patients and 109 age- and sex-matched controls were submitted to LEPs by the dorsum of the right hand and the skin over the right chest and knee tender point stimulation. Skin biopsy was performed in 21 randomly selected FM patients and 60 age- and sex-matched controls. The mean N2–P2 amplitude was reduced in the whole FM group, with normal or even increased values in patients with migraine as comorbidity and reduced values in other patients including those presenting with distal sensory deficits. All patients had reduced N2–P2 habituation in respect to controls. In the FM group, LEPs habituation was correlated with pain at tender points and bad quality of life. Epidermal fiber density was significantly reduced in FM patients versus controls, and correlated with N2–P2 amplitude by the hand and chest tender-point stimulation. Dysfunction in the nociceptive system at both the central and peripheral levels may concur to explain phenotypical eterogeneity and clinical symptom complexity in fibromyalgia.

Keywords Fibromyalgia · Laser-evoked potentials · Skin biopsy · Peripheral and central nervous system dysfunction

M. de Tommaso (✉) · E. Vecchio · K. Ricci · M. Lorenzo · M. Delussi · F. Girolamo · V. Lavolpe · P. Livrea
Basic Medical Sciences, Neuroscience and Sensory System Department, SMBNOS, Policlinico General Hospital, Bari Aldo Moro University, Via Amendola 207 A, 70124 Bari, Italy
e-mail: m.detommaso@neurol.uniba.it

M. Nolano · V. Provitera · A. Stancanelli
Neurology Department, "Salvatore Maugeri" Foundation IRCCS-Medical Center of Telese, Benevento, Italy

F. Iannone · G. Lapadula
Rheumatology Unit, Medical School, University of Bari Aldo Moro, Bari, Italy

Introduction

Fibromyalgia (FM) is a chronic disorder characterized by widespread pain and tenderness on palpation. The associated symptoms, identified by new diagnostic criteria, include non-restorative sleep, fatigue, and cognitive dysfunction [1]. FM affects up to 5 % of the general population worldwide and is associated with high medical and social costs [2]. The pathophysiology of FM remains largely unknown, however, an increase in central sensitization phenomena, probably based on abnormal pain modulation, is recognized in fibromyalgia as well as in other 'centrally driven' chronic pain syndromes [3]. There is increased activity of cortical regions devoted to pain processing, which has been suggested by neuroimaging studies [4]. Few studies were employed by laser-evoked potentials (LEPs), which are a specific tool for investigation of nociceptive pathways [5]. These studies confirmed increased responses from cortical zones devoted to noxious stimuli processing [6–9]. In addition, a pattern of reduced habituation under repetitive painful stimulation emerged in FM patients [9], which seems to characterize chronic pain syndromes subtended by enhanced phenomena of central sensitization such as migraine [10–12]. However, it has recently been found that peripheral factors may contribute

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | LE | TYPESET
MS Code : JOON-D-13-01229 | CP | DISK

to the abnormal activity of receptors in deep tissues [13], and a recent study employing skin biopsy and evoked responses obtained by concentric electrode (PREPs) in a cohort of 25 FM patients found that despite normal neurological and standard neurophysiological examination, excluding large-fiber polyneuropathy, both PREPs and skin biopsy suggested small afferents dysfunction [14]. These findings are in disagreement with the pattern of increased LEP amplitude previously described [9] and may suggest phenotypic heterogeneity among FM patients. So far, further information should be useful about nociceptive pathway functions at both the peripheral and central level in patients with fibromyalgia. In the present study, the aims were (1) to compare laser-evoked potentials features, including habituation, between a large cohort of FM patients and a group of healthy, age- and sex-matched subjects (2) to correlate LEPs features with clinical aspects of FM and (3) to report skin biopsy findings performed in a randomly selected sub-group of FM patients.

Methods

Subjects

We considered 370 consecutive out-patients between the ages of 18 and 65 for inclusion in the study. The patients visited the Neurophysiopathology of Pain Unit of the Bari Policlinico General Hospital between January 2, 2009, and December 20, 2012, after diagnosis of fibromyalgia was done in the Rheumatologic Clinic of the Bari Policlinico General Hospital in accordance with Wolfe et al. criteria [15]. The exclusion criteria were scholar age of less than 8 years, any peripheral or central nervous system (CNS) diseases, including spinal cord diseases and radiculopathies, diabetes, active thyroid insufficiency, renal failure, auto-immune diseases, active inflammatory arthritis, systemic connective tissue disease, present or previous history of cancer, as well as use of drugs acting on the CNS or chronic opioid therapy. Patients with primary headaches (see below) were admitted into the study. Patients taking analgesics were instructed to avoid analgesic use for 24 h prior to the laser-evoked potentials examination in order to avoid any effect on LEPs amplitudes [16]. Patients selected for the study were assigned to CNS-acting drug treatments only after both LEPs and clinical assessment were carried out.

There were 220 FM patients who met the inclusion criteria and subsequently submitted to neurophysiological examination. All of these patients also satisfied the recent diagnostic criteria [1]. Patients were also randomized 1–10 to be submitted to skin biopsy on the basis of the diagnosis of FM according to the ACR criteria [1, 15], without taking into consideration any other clinical features. The reason for the randomization was the availability of the procedure for a limited number of cases. The LEPs from 199 patients were included in the statistical analysis. The LEPs from the remaining 21 patients were incomplete recordings and not included in the final analysis. All the LEPs from patients submitted to skin biopsy were eligible for the analysis and statistical comparison.

There were 109 age- and sex-matched controls who were recruited among the patients' families, hospital staff, and students. The control subjects did not have symptoms or a history of any neurological, psychiatric, or general medical disorders, including migraine [17], and no history of CNS-acting drugs taken in the previous 3 months and/or analgesic use in the previous 24 h prior to the neurophysiological examination.

All subjects were informed about the purpose and the procedure of the study, for which they gave their consent. The study was approved by the Ethics Committee of the Bari Policlinico General Hospital.

Clinical examination

All patients were submitted to careful interview and standard neurological examination, including thorough bedside sensory testing. Since migraine is a comorbid condition [18, 19] and migraine may be a factor facilitating reduced LEPs habituation [10], we included a subgroup of patients with migraine. Migraine was defined as migraine without aura, migraine with aura, and chronic migraine, as defined by the International Headache Society (IHS) [17]. We recorded migraine patients in the intercritical period (at least 72 h after and 48 h before an attack, determined by a telephone interview). FM patients completed self-submitting scales exploring anxiety, depression [20, 21], fibromyalgia-linked invalidity [22], and quality of life [23] in accordance with previous studies [18, 19]. A psychologist explained the questionnaire scales and modalities of the responses to all participants. The tender point survey was used to measure the level of pain at any tender point [24].

Nerve conduction studies

Nerve conduction studies were performed according to standard methods [25]. The nerve conduction velocity was calculated and the compound action potential amplitude was measured for right sensory (sural) and posterior tibial nerve. We determined whether individual subjects data were within the range of normative reference values from our laboratory (antidromic sural nerve sensory nerve action potential amplitude ≥ 10 μV, sural nerve conduction velocity ≥ 42 m/s for all ages; tibial nerve compound

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

motor action potential ≥ 10 mV, tibial nerve conduction velocity ≥ 40 m/s for all ages).

Laser-evoked potentials-Recording procedure. Each subject was seated in a comfortable position, in a quiet room with an ambient temperature of 21–23 °C, in an awake and relaxed state with their eyes closed. All subjects and observers wore protective goggles during data acquisition. All subjects underwent a recording session with scalp electrodes placed over the Fz, Cz, and Pz positions of the 10–20 International System (impedance below 5,000 Ω), referring to the nasion with the ground at Fpz and by T3 and T4 derivation, referred to the Fz position. Another electrode was placed above the right eye to record the electrooculogram. The signals were amplified and stored on a biopotential analyzer (MICROMED System Plus).

Stimulation procedure

The stimulation site was visualized by an He–Ne laser beam. After each stimulation, the laser beam was slightly shifted to a nearby spot to avoid nociceptor sensitization and skin damage.

The pain stimulus consisted of laser pulses (wavelength 10.6 lm) that were generated by a CO_2 laser (Neurolas Electronic Engineering, Florence, Italy). The diameter of the laser beam was 2.5 mm, and the duration of the stimulus pulse was 30 ms. In order to define the pain threshold, single stimulus pulses were presented in random order at 4–5 different intensities with 1.5-W steps. The subjects were requested to report the quality of sensation and the perception threshold for each subject was represented at the different stimulation sites by the laser intensity (expressed in Watts) that produced a warm sensation while the pain threshold was the laser intensity that produced a pinprick sensation followed by a burning sensation. Three series of ten laser stimuli were then delivered at any stimulation site, at an intensity level, at two steps (3 W) above the pain threshold, with an inter-stimulus interval of 10 s, and an inter-series interval of 1 min. The dorsum of the right hand was stimulated in all patients and controls. In addition, we stimulated the skin over the tender points at the right knee and between the clavicle and the first rib, according to clinical feature of fibromyalgia [24]. These tender points were painful in all stimulated subjects and not painful in controls. The chest tender point was stimulated in 141 patients and 80 controls and the knee tender point in 60 patients and 30 controls. In cases where more than one site was stimulated, the order of site stimulation was randomized. We choose to stimulate only one side, to avoid a long and uncomfortable procedure.

Both patients and controls were requested to pay attention to the stimuli. At the end of each stimulation series, all subjects were requested to rate the pain induced by the laser stimuli using a 0–100 visual analogue scale (VAS) where 0 indicated no pain (white) and 100 (red) indicated the most severe pain imaginable.

Laser-evoked potentials analysis. An investigator who was blinded to the clinical condition analyzed the LEP recordings for 1 s, with a 100-ms pre-stimulus time, at a sampling rate of 256 Hz. All LEP recordings containing transient signals that exceeded 65 mV on any recording channel were excluded from the average by an automatic

Table 1 Clinical features of fibromyalgia patients and controls AQ3

	Fibromyalgia patients		Controls
Hand stimulation (*n*)	199		109
Sex	171 F; 28 M		89 F; 20 M (Chi square 1.2 n.s)
Age	40.55 ± 10.5		40.32 ± 9.99 (ANOVA *F* 1.22 n.s.)
Knee stimulation (*n*)	60		30
Sex	50 F; 10 M		25 F; 5 M (Chi square 1.1 n.s)
Age	38.8 ± 11.2		37.9 ± 12.2 (ANOVA *F* 0.89 n.s.)
Thorax stimulation (*n*)	141		80
Sex	125 F; 15 M		74 F; 6 M (Chi square 2.2 n.s)
Age	41.1 ± 9.9		40.9 ± 10.5 (ANOVA F 2.3 n.s.)
Presence of distal sensory deficit (*n*)	35 yes	164 no	
Age	51.11 ± 11	44.2 ± 13.4	
	(ANOVA *F* 9.52, *p* **0.02**)		
Sex	28 F; 7 M	150 F; 14 M	
	(Chi square: 1.79 n.s.)		
Presence of migraine (migraine with aura, without aura, chronic migraine—IHS) (*n*)	79 yes	120 no	
Age	43.67 ± 12.9	47.7 ± 13	
	(ANOVA *F* 33.4, $p < \mathbf{0.00001}$)		
Sex	67 F 3 M	94 F 26 M	
	(Chi square: 10.5, *p* **0.005**)		

Results of statistical analysis between groups are reported. For one-way ANOVA test, the degree of freedom (*df*) was 1

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

Table 2 Mean values and standard deviation of laser pain threshold and sensation and laser evoked potential parameters in fibromyalgia patients and controls

Hand stimulation	Fibromyalgia patients ($n = 199$)	Controls ($n = 109$)	ANOVA
Laser pain threshold (Watts)	8.3 ± 4.2	7.9 ± 3.5	$F = 1.22$ n.s.
Laser pain sensation (VAS 0–100)	41.42 ± 23.6	41.19 ± 23.7	$F = 0.87$ n.s.
N1 latency (ms)	163.73 ± 20.7	168 ± 21.12	$F = 1.52$ n.s.
N1 amplitude (μV)	6.89 ± 4.57	6.76 ± 5.52	$F = 0.12$ n.s.
N2 latency (ms)	219.32 ± 32.13	229.33 ± 31.13	$F = 0.77$ n.s.
P2 latency (ms)	335.85 ± 44.12	346.23 ± 29.9	$F = 0.81$ n.s.
N2–P2 amplitude (μV)	16.36 ± 10.11	21.0 ± 12.23	$F = 10.68$, $p =$ **0.0012**
Thorax stimulation	Fibromyalgia patients ($n = 141$)	Controls ($n = 80$)	
Laser pain threshold (Watts)	6.9 ± 3.9	6.7 ± 4.2	$F = 0.89$ n.s.
Laser pain perception (VAS 0–100)	44.61 ± 26.4	30.19 ± 18.6	$F = 3.55$, $p = 0.061$ n.s.
N1 latency (ms)	163.61 ± 22.7	160 ± 11.12	$F = 1.1$ n.s.
N1 amplitude (μV)	6.91 ± 4.51	8.54 ± 5.0	$F = 1.34$ n.s.
N2 latency (ms)	211.3 ± 42.11	209.45 ± 29.13	$F = 0.65$ n.s.
P2 latency (ms)	328.15 ± 33.45	336.18 ± 34.9	$F = 0.67$ n.s.
N2–P2 (μV)	18.05 ± 27.5	23.05 ± 12.2	$F = 0.79$ n.s.
Knee stimulation	Fibromyalgia patients ($n = 60$)	Controls ($n = 30$)	
Laser pain threshold (Watts)	7.5 ± 2.8	7.9 ± 2.9	$F = 0.45$ n.s.
Laser pain perception (VAS 0–100)	52.24 ± 2.4	50.55 ± 20.2	$F = 0.021$ n.s.
N1 latency (ms)	173.61 ± 22.7	170 ± 11.12	$F = 1.1$ n.s.
N1 amplitude (μV)	4.89 ± 6.9	5.9 ± 3.2	$F = 0.13$ n.s.
N2 latency (ms)	243.3 ± 44.11	252.45 ± 25.16	$F = 0.58$ n.s.
P2 latency (ms)	365.15 ± 43.45	367.13 ± 29.9	$F = 0.47$ n.s.
N2–P2 (μV)	12.93 ± 7.9	20.24 ± 14.31	$F = 3.98$, $p =$ **0.049**

All values were corrected for age. The one-way ANOVA results are reported (*df* 1)

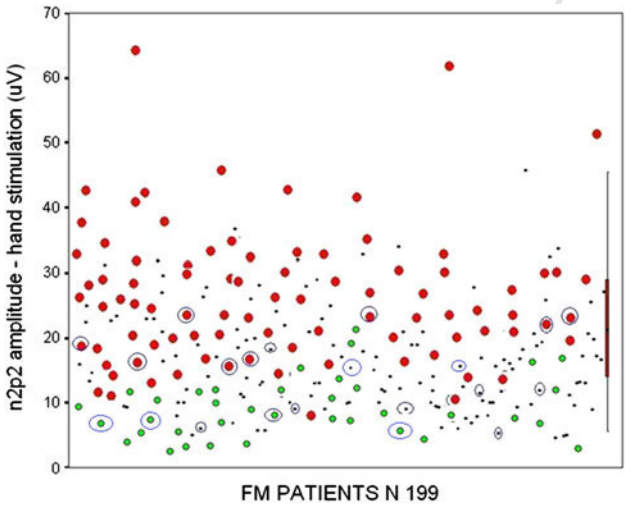

Fig. 1 The values of N2–P2 amplitudes by hand stimulation are depicted for single fibromyalgia cases ($n = 199$). The *box blot* represents values (95 % confidence interval) from controls ($n = 109$). Patients with migraine comorbidity are outlined in *red* and patients with sensory deficits are outlined in *green*. Patients subjected to skin biopsy are indicated with the *blue ellipsis*. Data were corrected for age

artifact rejection algorithm. Other artifacts were visually inspected. For each stimulation site (right hand, right thorax, and right knee), an average was obtained across each series of stimuli. The LEPs were identified on the basis of their latency and distribution and three responses (N1, N2, and P2) were labeled according to the procedure of Valeriani et al. [26]. The N1 component was analyzed at T3-Fz and the N2 and P2 components were analyzed at the vertex (Cz). 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, and the baseline was measured automatically by calculating the average signal on the whole sweep and subtracting it from the trace (ASA-v.4.6 by ANT software; Advanced Neuro Technology, Enschede, The Netherlands). The peak-to-peak amplitude was taken into consideration for the vertex biphasic LEP component (N2–P2). To assess the LEP habituation, the quotient between the LEP amplitudes obtained in the third and the first block of evoked responses was computed. This was termed the habituation index (HI).

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | LE | TYPESET
MS Code : JOON-D-13-01229 | CP | DISK

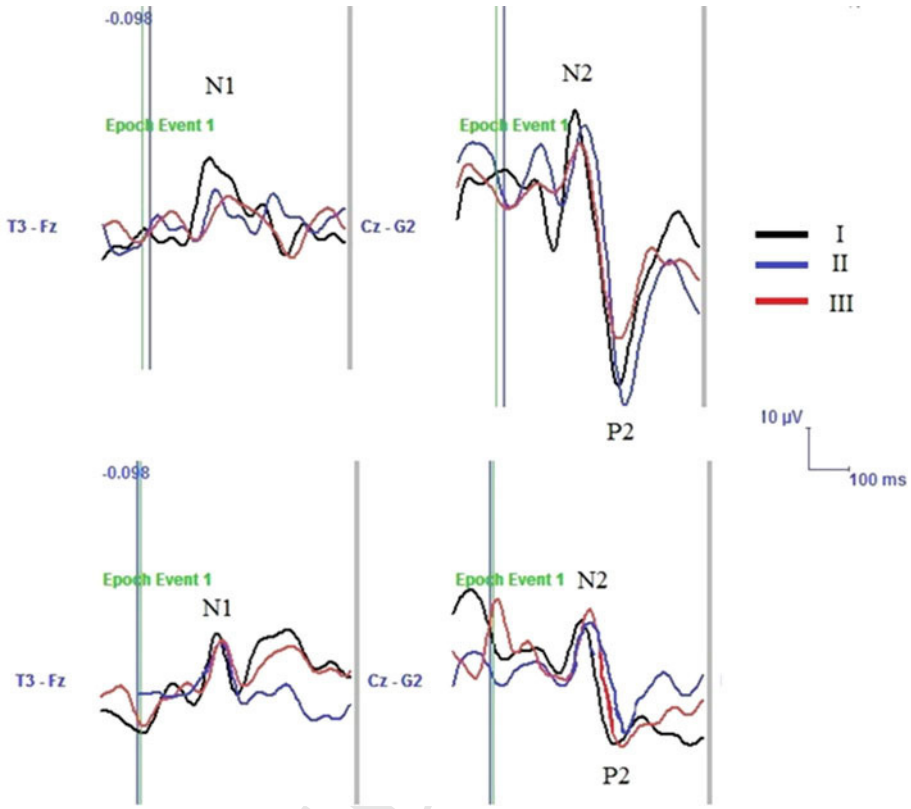

Fig. 2 Laser-evoked potentials (LEPs) from representative cases are shown. A control female (*top panel*) and a female affected by fibromyalgia and distal sensory deficit (*bottom panel*) are shown. Both are 21 years old. The averages across three consecutive repetitions are shown

Table 3 Mean values and standard deviation of the habituation index (HI) in fibromyalgia patients and controls

Habituation index N2–P2	FM patients	Controls	ANOVA (*df* 1)
Hand	1.29 ± 1.71	0.68 ± 2.73	F = 4.75
	(n = 199)	(n = 109)	p = 0.03
Chest	2.39 ± 3.45	0.75 ± 2.37	F = 4.06
	(n = 141)	(n = 80)	p = 0.045
Knee	1.24 ± 2.18	0.67 ± 2.89	F = 4.92
	(n = 60)	(n = 30)	p = 0.029

All values were corrected for age. The one-way ANOVA results are reported (*df* 1)

Skin biopsy

In 21 patients and in 60 healthy subjects, age and sex matched, 3-mm punch biopsies were taken from fingertip (V digit), thigh, and leg after intradermal injection of 1 % Xylocaine. Samples were fixed overnight in Zamboni solution, cut in 50-μm sections using a freezing slide microtome (Leica) and processed using indirect immunofluorescence techniques as described [27]. Briefly, to mark neural and vascular structures, free-floating sections were incubated with a panel of primary antibodies (Table 1) and then with secondary antibodies labeled with Cy2–Cy3–Cy5 fluorophores to visualize the antigens. Sections were then fixed on coverslips with agarose, dehydrated in 95 and 100 % ethyl alcohol, clarified in methyl salicylate, and finally mounted in DPX. Quantification of epidermal nerve fibers was performed on four nonconsecutive PGP-Col IV double-stained sections following previously described procedures [27]. Intrapapillar myelinated endings and Meissner corpusclets in glabrous skin sections were counted on alternate sections and density calculated as number of structures/area as previously described [27].

Statistical analysis

LEPs features obtained at the three sites, including the habituation index, were compared between patients and controls by one-way ANOVA, with diagnosis as factor. In addition, we compared the amplitude and habituation of the N2P2 complex, obtained in the three consecutive series of laser stimulation at the hand, across patients with sensory deficits, patients with migraine, patients without migraine and sensory deficits, and controls by one-way ANOVA with the post hoc Bonferroni test. In statistical comparisons, a correction for age was applied to LEPs' amplitude and latencies, in accordance with the results of Truini et al. [28]. In the FM group, LEPs by hand stimulation were correlated with clinical features using the Spearman correlation test. In the patients submitted to skin biopsy,

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

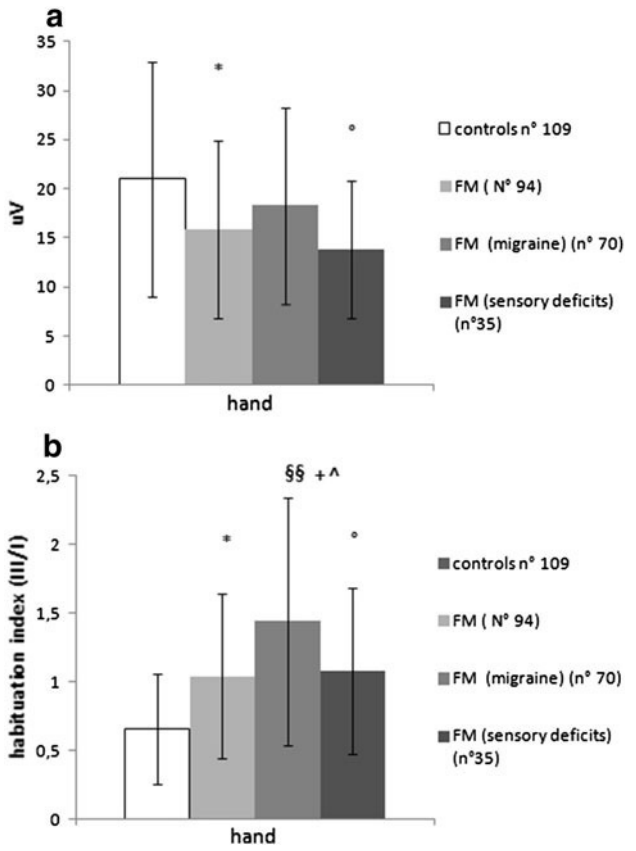

Fig. 3 **a** Mean values and standard deviations of N2–P2 complex obtained by the right hand in fibromyalgia (FM) patients subgroups and controls. Values were corrected for age. The one-way ANOVA results with groups as factor were: $F = 9.33$, $p = 0.02$. Results of Bonferroni test are reported: *asterisk* indicates controls versus FM: $p < 0.05$° controls versus FM patients with sensory deficit: $p < 0.01$. **b** Mean values and standard deviations of N2–P2 habituation index in fibromyalgia (FM) patients subgroups and controls. Values were corrected for age. The one-way ANOVA results with groups as factor were: $F = 9.88$, $p = 0.018$. Results of Bonferroni test are reported: *asterisk* indicate controls versus FM: $p < 0.05$° controls versus FM patients with sensory deficit: $p < 0.01$; controls versus FM migraine: $^{\S\S}p < 0.01$; FM migraine versus FM patients: $^{+}p < 0.05$; FM migraine versus FM with sensory deficit: $^{\wedge}p < 0.05$

Student's *t* test was applied to compare skin biopsy data between patients and controls. The ENF density was also correlated with LEPs amplitudes and main clinical features by means of Spearman's correlation test. In all considered statistical tests, a *p* value < 0.05 was considered as significant.

Results

Clinical features

A minority of patients (17.5 %) presented with distal sensory deficits at the standard clinical assessment. These deficits consisted of slightly reduced pinprick and thermal sensation at the feet in all patients, with slight sensory deficits involving also the hands in two cases (Table 1). The clinical syndrome of these patients confirmed the ACR criteria [1, 15]. These patients were significantly older in respect to patients without signs of sensory deficits (Table 1). A large number of FM patients presented with migraine (39.79 %, Table 1). The migraine patients were younger and female patients were more prevalent (Table 1).

Nerve conduction study

In FM patients, both motor and sensory nerve conduction velocities and action potential amplitudes were within normal limits. Also, patients reporting distal sensory deficit presented with sural sensitive potential amplitude within the normal ranges.

Laser-evoked potentials

The laser pain threshold and the subjective pain sensation were similar between patients and controls for all the stimulation sites. However, a slight increase in pain sensation was observed in FM patients compared to controls when the skin over the chest tender point was stimulated, which approached statistical significance (Table 2). The N1 amplitude was similar among patients and controls, as well as N1, N2, and P2 latencies (Table 2). The vertex N2–P2 complex amplitude was significantly reduced in FM patients compared to controls when the hand and the knee were stimulated, whereas when the skin over the chest tender point was stimulated, there was a non-significant reduction in the FM group compared to control one (Table 2) (Figs. 1, 2).

The N2P2 habituation index (HI) was significantly increased in FM patients compared to controls when all the stimulation sites were considered (Table 3). The N2–P2 habituation index was not significantly correlated to N2–P2 amplitude (Spearman correlation test: hand 0.34 n.s; chest tender point 0.98 n.s.; knee 1.12 n.s).

LEPs and clinical features

Patients presenting with migraine did not display significant N2–P2 amplitude decrease when the hand was considered, differently from patients with sensory deficits and the remaining FM sufferers (Fig. 3a). Habituation index was significantly increased in all FM groups, in respect to controls. Fibromyalgia patients with migraine comorbidity showed even potentiation of LEPs amplitude in the third repetitions, so habituation index was incremented also when compared to other FM groups (Table 3; Fig. 3b).

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

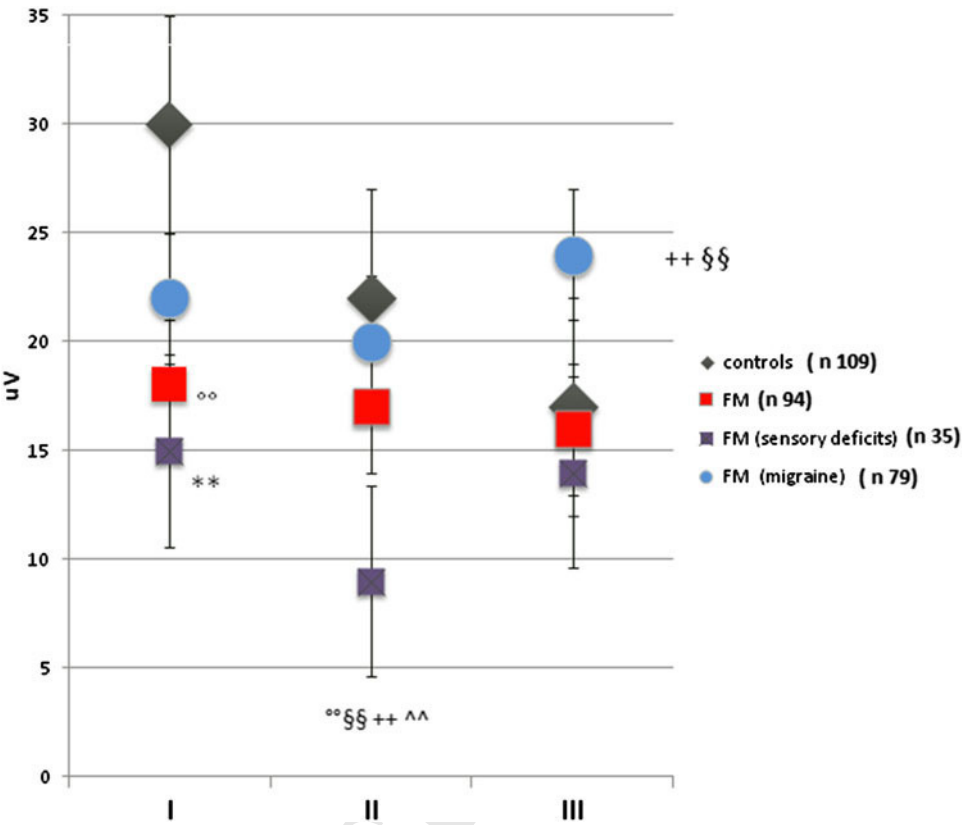

Fig. 4 The mean value and standard error of N2–P2 amplitude by hand stimulation across three consecutive repetitions are shown for FM patient subgroups and controls. For the first repetition, the ANOVA test was 3.86, *df* 3, $p = 0.01$; for the second repetition the ANOVA test was 2.66, $p = 0.048$; and for the third repetition the ANOVA test was 1.86, n.s. The results of the Bonferroni test for multiple comparisons are as follows: fibromyalgia with sensory deficit versus controls, $^{**}p < 0.01$, $^{*}p < 0.05$; fibromyalgia with migraine versus controls, $^{++}p < 0.01$; fibromyalgia without sensory deficit and migraine versus controls, $^{\circ\circ}p < 0.01$; fibromyalgia with migraine versus fibromyalgia with sensory deficit, $^{\S}p < 0.05$; $^{\S\S}p < 0.01$. All data were corrected for age

Considering the LEPs by hand stimulation across the three consecutive series, FM patients, excluding those with migraine, exhibited significant reduced amplitude in the first series in comparison with controls. All patient subgroups showed a tendency towards amplitude increase in the third series, especially migraine patients for whom this was statistically relevant compared to controls and other FM subgroups (Fig. 4).

There was a positive correlation between hand habituation index and pain at tender points (Spearman correlation test 0.329, $p < 0.01$) and a negative correlation between the habituation index and physical component of quality of life (0.346, $p < 0.01$) (Fig. 5).

Skin biopsy

Demographic and clinical data, LEP amplitudes, and quantitative analysis of cutaneous sensory nerve endings of patients randomized for skin biopsy are summarized in Tables 4 and 5. Eight patients presented with migraine comorbidity and four with distal sensory deficit (Table 5).

In FM patients, we found a significant non length-dependent loss of epidermal nerve fibers (ENF) in thigh, leg, and fingertip, compared to the age- and sex-matched control group (Tables 4, 5).

Moreover, there was a significant loss of Meissner corpuscles, while intrapapillar myelinated fibers appeared speared.

Sixteen out of 21 patients had ENF density below the 5° percentile cut-off in at least one site and 13 of them had abnormal values also for MC (Table 5). Of the remaining five, four showed only low values of MC density and one had normal values of densities for ENF, MC, and IMF.

ENF density at fingertip correlated with the N2–P2 complex amplitude obtained stimulating the hand ($n = 21$; Spearman's correlation test: 0.55, $p = 0.01$) and at chest tender point ($n = 20$; Spearman's correlation test: 0.524, $p = 0.018$). The ENF density was not correlated with habituation index as well as with any clinical feature, including pain at tender point.

Discussion

Laser-evoked potential features

This study confirmed only in part previous results obtained in a small group [9]. The increase in case series allowed us to observe a wide distribution of FM patients with regard to LEP's amplitude in comparison to controls, from patients showing increased amplitude to patients presenting with

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | LE | TYPESET
MS Code : JOON-D-13-01229 | CP | DISK

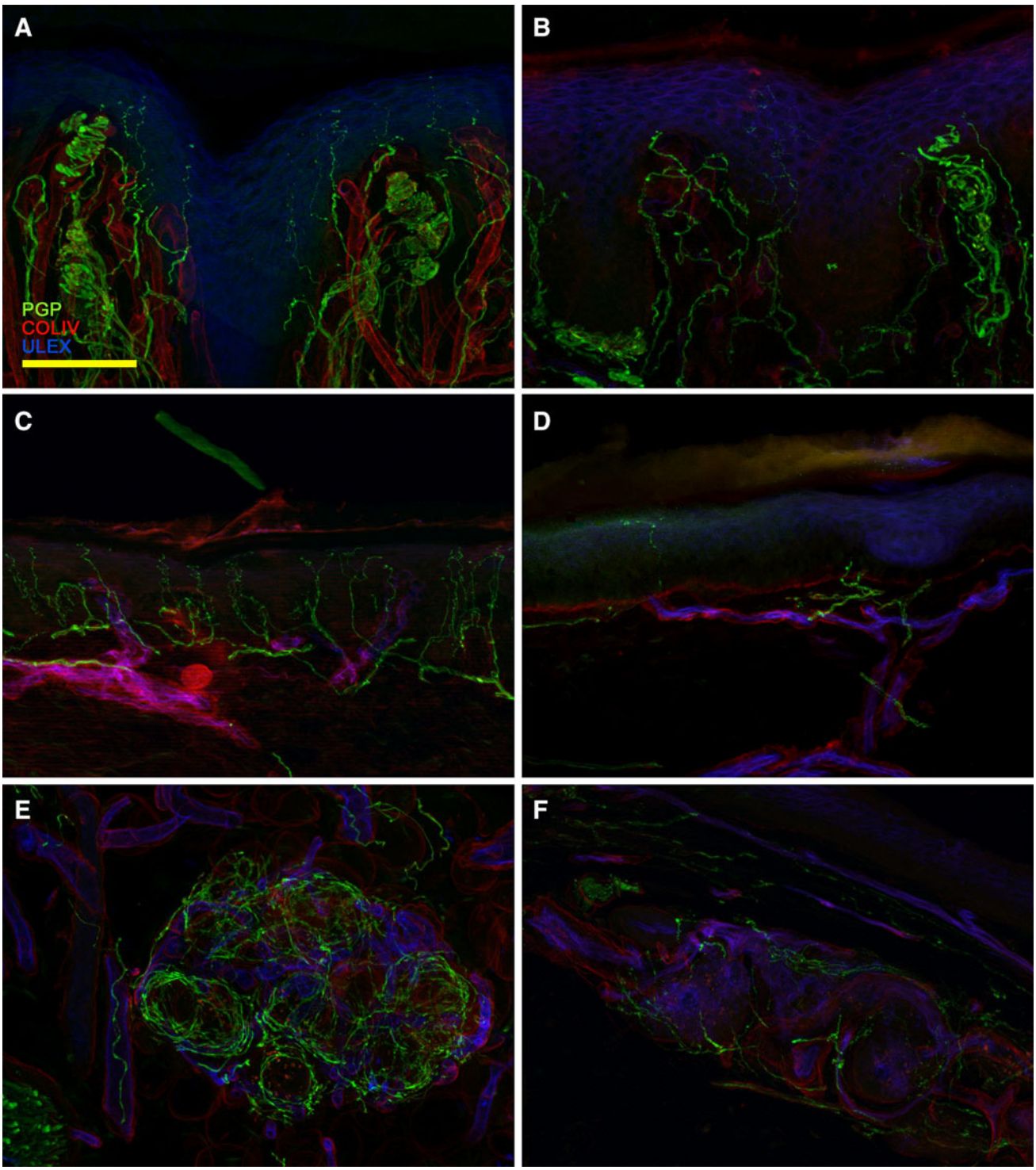

Fig. 5 Confocal images of sensory and autonomic innervation in a patient with fibromyalgia (**b**, **d**, **f**) compared to a healthy control (**a**, **c**, **e**). Nerve fibers are in *green* (PGP), blood vessels and basal membrane are in *red* (Col IV), epidermis and endothelia are in *blue* (ULEX). In **b** compared to **a**: a loss of Meissner corpuscles and epidermal nerve fibers is evident in patient fingertip. In **d** compared to **c**: a severe loss of epidermal nerve fibers with a poor subepidermal neural plexus is present in the patient leg. In **f** compared to **e**: there is a severe loss of sudomotor nerves in patient leg compared to control. *Scale bar* 100 μm

decreased values of N2–P2 amplitude, while reduced habituation observed across three consecutive LEPs tracks was confirmed in almost all patients [9]. Uçeyler et al. [14] recently described an amplitude reduction and latency increase of the negative–positive vertex complex induced by concentric electrode stimulation in a small FM patient

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | ☐ LE | ☐ TYPESET
MS Code : JOON-D-13-01229 | ☑ CP | ☑ DISK

Table 4 Mean values and standard deviations of the number of epidermal nerve fibers (EFN) per linear mm, Meissner corpuscles (MC) per mm^2, and intrapapillar myelinated fibers (IMF) per mm^2

	Sex (M/F)	Age	EFN thigh	EFN leg	EFN fingertip	MC	IMF
Fibromyalgia	3/18	51.0 ± 8.7	17.4 ± 6.9	11.4 ± 4.3	4.5 ± 3.2	9.7 ± 8.3	59.5 ± 25.7
Controls	10/50	52.7 ± 6.3	23.5 ± 3.8	15.0 ± 3.6	6.8 ± 3.0	27.2 ± 7.5	53.1 ± 19.3
p		0.45	**<0.01**	**<0.01**	**<0.05**	**<0.01**	0.33

The results of Student's *t* test are reported

population compared to healthy subjects and patients with major depression. The authors postulated small fiber neuropathy taking into consideration the concurrence of nociceptive-evoked responses, quantitative sensory testing (QST), and skin biopsy results. Due to the different types of stimulation employed in the two studies, reliable comparisons of the neurophysiological findings are not possible [29]. However, the present results concur with that study [14] for the reduced amplitude of the negative–positive major deflection, while we failed to show latency prolongation of the negative component of the vertex complex. A proximal leg stimulation site was employed in this study, following typical pain distribution in FM syndrome [15], while in the study by Uçeyler et al. [14], a distal point on the foot was employed, with an increased probability to relieve a slowing in conduction along the nociceptive afferents.

We failed to observe amplitude reduction of the early N1 component in the FM patients. The laser-induced N1 was not previously examined in patients with painful sensory polyneuropathy [30], but it was found to be reduced in amplitude in patients with neuropathic pain by Fabry's disease [31]. Local morphologic alterations in the cingulate cortex and the insula, sparing cortical zones where the N1 originates [32], were detected in patients suffering from various chronic pain syndromes, including fibromyalgia [33], and may explain the selective preservation of N1 in FM patients. The N1 variability linked to its small amplitude [34] may also concur to explain the absence of significant abnormalities we observed in our patients. In our opinion, this finding may contribute to confirm the complexity of FM syndrome, where the concurrence of peripheral and central factors, as the dysfunction of small sensory fibers and cortical zones electively devoted to pain modulation, may account for the complex and apparently contradictory results. The pattern of reduced vertex LEPs is generally attributed to a lesion or disease of the somatosensory system causing neuropathic pain [35], while the FM patients included in this study were carefully selected for the absence of any central or peripheral nervous system disease. The patients in this study also displayed normal sensory-nerve conduction studies, which demonstrate the normal function of a-beta sensory fibers.

Habituation deficit across three consecutive LEPs repetitions seemed to be a constant pattern across FM patients. This was evident for all three stimulated sites, independent from total amplitude of the averaged LEPs. In fact, in many FM cases, the deficit of habituation did not result in a vertex complex amplitude increase, characterizing patients with incremented, normal, or reduced LEPs. Reduced habituation seemed to involve nociceptive-evoked responses in migraine, fibromyalgia, and other conditions of uncertain origin [9–11], supporting the possible role of complex dysfunction of the endogenous antinociceptive system in these syndromes [36].

LEPs features and clinical aspects of FM

A careful neurological examination enabled us to find a small sub-group of FM patients with a slight distal sensory deficit and LEPs amplitude reduction, which on the other hand characterized also patients with normal neurological examination. Abnormalities of neurological examination might be absent in the majority of FM patients with reduced LEPs for different reasons as scarce compliance during the sensibility examination [37] or dysfunction of too limited extension to become evident unless a QST is performed. A possible dysfunction of nociceptive afferents in FM patients may also explain the absence of significant reductions in N2–P2 amplitude when a proximal site such as the chest tender point was stimulated. However, the phenotypic heterogeneity within FM patients is suggested by the normal or even increased LEPs exhibited by some patients, most of which being also migraine sufferers. Migraine patients for example constitute a sub-group of FM patients where the pronounced expression of central sensitization phenomena are widely accepted [10, 12, 38] and may sustain fibromyalgia syndrome in the absence or in cooperation of nociceptive afferent dysfunction.

We also observed that deficient habituation across LEPs repetitions characterized patients with slight sensory deficit and reduced total N2–P2 amplitude, who may be reliably affected by a peripheral involvement of nociceptive afferents. This finding may suggest that both impaired small fibers function and altered modulation of pain at the central

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

Table 5 Epidermal nerve fibers density in the fibromyalgia patients

Case	Site	Age	ENF/mm	N2–P2 (hand) (μV)	Migraine	Distal sensory deficit	Tender
1	Thigh	44	**12.8**	23.24	Yes	No	35
	Leg		**6.3**				
	V F		**1.7**				
2	Thigh	40	14.6	19	Yes	No	103
	Leg		24.8				
	V F		6.1				
3	Thigh	61	**6.3**	8.87	No	Yes	170
	Leg		**7.5**				
	V F		**2.9**				
4	Thigh	40	20.4	9.32	No	No	45
	Leg		10.2				
	V F		**2.8**				
5	Thigh	42	**10.7**	16	Yes	No	150
	Leg		**7.1**				
	V F		**3**				
6	Thigh	40	**14**	5.4	No	Yes	130
	Leg		**5.9**				
	V F		**0.1**				
7	Thigh	58	**11.7**	12.22	No	No	120
	Leg		10.8				
	V F		**4**				
8	Thigh	38	**17.1**	15.22	No	No	94
	Leg		18.7				
	V F		**4.1**				
9	Thigh	66	26.1	23.22	Yes	No	140
	Leg		16				
	V F		10.2				
10	Thigh	39	**12.2**	15.3	Yes	No	87
	Leg		**10.6**				
	V F		**2.3**				
11	Thigh	54	**8.5**	11.88	No	No	53
	Leg		**9.7**				
	V F		**3.3**				
12	Thigh	43	21.6	5	No	Yes	69
	Leg		27.2				
	V F		**0.7**				
13	Thigh	54	**12**	12.22	No	No	36
	Leg		**10.1**				
	V F		**4.4**				
14	Thigh	49	26	23.4	Yes	No	62
	Leg		15.7				
	V F		6.2				
15	Thigh	39	21.7	24.4	Yes	No	77
	Leg		14.4				
	V F		11.4				
16	Thigh	41	**13.4**	10.4	No	No	148
	Leg		13.5				
	V F		**5.5**				
17	Thigh	50	27.9	10	Yes	No	127
	Leg		12				
	V F		10.2				
18	Thigh	54	26.5	6	No	Yes	52
	Leg		n.e.				
	V F		**3.5**				
19	Thigh	61	**8.2**	8.8	No	No	50
	Leg		**5.6**				
	V F		**1.5**				
20	Thigh	51	22	11.27	No	No	34
	Leg		**9.6**				
	V F		**5.3**				
21	Thigh	52	**16**	15.59	Yes	No	35
	Leg		12.1				
	V F		8				

The N2–P2 amplitude by right-hand stimulation, the comorbidity for migraine, the presence of sensory deficit, pain at tender points score (Tender). The findings below the 5° percentile are reported in *bold*

VF V finger tip, *n.e.* not evaluable

level may coincide to determine the fibromyalgia syndrome in subgroups of patients. In patients with migraine as comorbidity, habituation deficit toward facilitation was even more evident compared to other FM patients. In patients with sensory deficit, the response of the first block was further reduced, in agreement with potential peripheral afferent dysfunction, but the recovery in the third response was not enough to reach normal LEPs amplitude.

The prevalence of sensitization on habituation seems to be a common feature across patients. It was also correlated with pain severity at tender points and poor quality of life due to impairment of physical condition. No correlation was found between LEPs habituation and anxiety and depression, which is in contrast to our previous report [9]. The tendency not to habituate across consecutive sessions of painful stimulation seems to be a stable pattern in fibromyalgia, which is not influenced by psychological factors and is correlated to illness severity and invalidity.

Skin biopsy features

We found a small fiber involvement in the majority of our patients that underwent skin biopsy. This finding is in

Journal : Large 415 Dispatch : 17-12-2013 Pages : 12
Article No. : 7211 LE TYPESET
MS Code : JOON-D-13-01229 CP DISK

agreement with the recent report by Uceyler et al. [14] demonstrating a reduction of ENF in fibromyalgia. However, the possibility to evaluate ENF density in multiple sites allowed to show a non length-dependence of this pathologic process and then to differentiate it by the classical small fiber neuropathy. In addition, we observed a significant loss of MC. This pathological aspect was present in 19 out of 21 patients, so a common pathogenic mechanism could induce the degeneration of last endings of large and small fibers. Therefore, this degenerative process cannot be revealed by sensory nerve conduction that cannot explore small fibers and by-passes the most distal part of large fibers. Our skin biopsy reports are not completely representative of the entire FM population because we were able to randomly submit to this procedure only a small sub-group of FM patients. However, the FM characteristics we decided to individuate in the entire group were represented among patients submitted to skin biopsy, so we can suppose that a dysfunction of sensory afferents may be a common factor of the disease, involving also some cases with associated migraine. We found a positive correlation between N2 and P2 amplitude by hand and chest stimulation and ENF density at the fingertip, with lack of correlation between skin biopsy data, habituation index, and pain at tender points. This may confirm that the clinical manifestation of FM is correlated to the dysfunction of pain modulation as expressed by the relationship observed with LEPs habituation, more than to the possible peripheral sufferance of nociceptive afferents. In fact, in FM patients, symptoms of peripheral afferents involvement are different from those displayed by patients with classical small fiber neuropathy [39–41]. In addition, our cases were carefully selected in order to exclude metabolic, endocrine, immune, and neoplastic diseases, which frequently subtend small fiber sufferance [40]. The reduction of LEPs from the hand dorsum seems also non-typical for patients with classical small fiber neuropathy [40], as well as the lack of length-dependent ENF loss. Our present opinion, which needs further confirmation by the enlargement of the skin biopsy data, is that idiopathic peripheral sensory nerve involvement may be part of FM syndrome, with clinical and pathological features different from other syndromes as the classical small fiber neuropathy [39, 40].

Conclusions

The present results confirmed the complexity of FM syndrome. The possible involvement of sensory afferents may be present in most FM patients, as shown by LEPs' amplitude reduction found in a large cohort of patients and reduced ENF density observed in a restricted FM group. Moreover, reduced habituation in the course of laser stimulation may express a central mechanism of altered pain modulation, which correlated with the clinical appearance of fibromyalgia. This may justify previous findings on LEPs in FM [6–9] giving that reduced habituation may compensate an initial gap in nociceptive input. Further skin biopsy data are needed to confirm the sufferance of sensory fibers as a common feature in fibromyalgia. Our patients were carefully selected for the absence of factors that may cause polyneuropathies, so the small fibers involvement would be idiopathic and probably extended to muscle and joint afferents [13]. Abnormalities of ionic channels may explain altered neuronal excitability [41–43], evolving toward neuronal degeneration at both central [33] and peripheral levels [44]. Peripheral sensitization of axon terminals increases the expression of sodium channels that in turn could lead to axonal remodeling and degeneration [45].

In light of the present results, we can suppose that in FM a phenotypical heterogeneity may be based on a different balance of central versus peripheral factors in the different patients.

Conflict of interest No author declares a conflict of interest. AQ6

References

1. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB (2010) The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care Res (Hoboken) 62(5):600–610
2. White KP, Harth M (2001) Classification, epidemiology and natural history of fibromyalgia. Curr Pain Headache Rep 5:320–329
3. Phillips K, Clauw DJ (2011) Central pain mechanisms in chronic pain states—may be it is all in their head. Best Pract Res Clin Rheumatol 25(2):141–154
4. Staud R (2011) Brain imaging in fibromyalgia syndrome. Clin Exp Rheumatol 29(6 Suppl 69):S109–S117
5. Carmon A, Mor J, Goldberg J (1976) Evoked cerebral responses to noxious thermal stimuli in humans. Exp Brain Res 25:103–107
6. Gibson SJ, Littlejohn GO, Gorman MM, Helme RD, Granges G (1994) Altered heat pain thresholds and cerebral following painful CO_2 laser stimulation in subjects with fibromyalgia syndrome. Pain 58(2):185–193
7. Lorenz J, Grasedyck K, Bromm B (1996) Middle and long latency somatosensory evoked potentials after painful laser stimulation in patients with fibromyalgia syndrome. Electroencephalogr Clin Neurophysiol 100:165–168
8. Lorenz J (1998) Hyperalgesia or hypervigilance? An evoked potential approach to the study of fibromyalgia syndrome. Z Rheumatol 57:19–22
9. de Tommaso M, Federici A, Santostasi R, Calabrese R, Vecchio E, Lapadula G, Iannone F, Lamberti P, Livrea P (2011) Laser-evoked potentials habituation in fibromyalgia. J Pain 12(1):116–124
10. Valeriani M, de Tommaso M, Restuccia D, Le Pera D, Guido M, Iannetti GD, Libro G, Truini A, Di Trapani G, Puca F, Tonali P, Cruccu G (2003) Reduced habituation to experimental pain in

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | LE | TYPESET
MS Code : JOON-D-13-01229 | CP | DISK

migraine patients: a CO(2) laser evoked potential study. Pain 105(1–2):57–64
11. Valeriani M, Sestito A, Le Pera D, De Armas L, Infusino F, Maiese T, Sgueglia GA, Tonali PA, Crea F, Restuccia D, Lanza GA (2005) Abnormal cortical pain processing in patients with cardiac syndrome X. Eur Heart J 26(10):975–982
12. Yunus MB (2007) Fibromyalgia and overlapping disorders: the unifying concept of central sensitivity syndromes. Semin Arthritis Rheum 36(6):339–356
13. Staud R (2011) Peripheral pain mechanisms in chronic widespread pain. Best Pract Res Clin Rheumatol 25(2):155–164
14. Uçeyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C (2013) Small fibre pathology in patients with fibromyalgia syndrome. Brain 36(Pt 6):1857–1867
15. Wolfe F, Smythe HA, Yunus MB, Bennet RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, Fam AG, Farber SJ, Fiechtner JJ, Franklin CM, Gatter RA, Hamaty D, Lessard J, Lichtbroun AS, Masi AT, McCain GA, Reynolds WJ, Romano TJ, Russel IJ, Sheon RP (1990) The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the multicenter criteria committee. Arthritis Rheum 33:160–172
16. Truini A, Panuccio G, Galeotti F, Maluccio MR, Sartucci F, Avoli M, Cruccu G (2010) Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. Eur J Pain 14(2):222–225
17. Headache Classification Committee (2004) The international classification of headache disorders II. Cephalalgia 24:24–136
18. de Tommaso M, Sardaro M, Serpino C, Costantini F, Vecchio E, Prudenzano MP, Lamberti P, Livrea P (2009) Fibromyalgia comorbidity in primary headaches. Cephalalgia 29(4):453–464
19. de Tommaso M, Federici A, Serpino C, Vecchio E, Franco G, Sardaro M, Delussi M, Livrea P (2011) Clinical features of headache patients with fibromyalgia comorbidity. J Headache Pain 12(6):629–638
20. Zung WWK (1965) A self-rating depression scale. Arch Gen Psychiatry 12:63–70
21. Zung WWK (1976) SAS, Self-Rating Anxiety Scale. In: Guy W (ed) ECDEU assessment manual for psychopharmacology, revised edn. National Institute of Health, Psycho-pharmacology Research Branch, Rockville, MD, pp 337–340
22. Burckhardt CS, Clark SR, Bennett RM (1991) The Fibromyalgia Impact Questionnaire (FIQ): development and validation. J Rheumatol 18:728–733
23. Ware JE, Kosinski M, Dewey JE (2000) How to score version 2 of the SF-36 (r) health survey. Quality Metric Incorporated, Lincoln, RI
24. Starz TW, Sinclair JD, Okifuji A (1997) Putting the finger on fibromyalgia: the manual tender point survey. J Musculoskel Med 17:61–67
25. Kimura J (2001) Electrodiagnosis in diseases of nerve and muscle: principles and practice. Oxford University Press, New York
26. Valeriani M, Rambaud L, Mauguiere F (1996) Scalp topography and dipolar source modelling of potentials evoked by CO_2 laser stimulation of the hand. Electroencephalogr Clin Neurophysiol 100:343–353
27. Nolano M, Provitera V, Estraneo A, Selim MM, Caporaso G, Stancanelli A, Saltalamacchia AM, Lanzillo B, Santoro L (2008) Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation. Brain 131(Pt 7):1903–1911
28. Truini A, Galeotti F, Romaniello A, Virtuoso M, Iannetti GD, Cruccu G (2005) Laser-evoked potentials: normative values. Clin Neurophysiol 116:821–826
29. de Tommaso M, Santostasi R, Devitofrancesco V, Franco G, Vecchio E, Delussi M, Livrea P, Katzarava Z (2011) A comparative study of cortical responses evoked by transcutaneous electrical versus CO(2) laser stimulation. Clin Neurophysiol 122(12):2482–2487
30. Casanova-Molla J, Grau-Junyent JM, Morales M, Valls-Solé J (2011) On the relationship between nociceptive evoked potentials and intraepidermal nerve fiber density in painful sensory polyneuropathies. Pain 152(2):410–418
31. Valeriani M, Mariotti P, Le Pera D, Restuccia D, De Armas L, Maiese T, Vigevano F, Antuzzi D, Zampino G, Ricci R, Tonali P (2004) Functional assessment of a delta and C fibers in patients with Fabry's disease. Muscle Nerve 30(6):708–713
32. Garcia-Larrea L, Frot M, Valeriani M (2003) Brain generators of laser-evoked potentials: from dipoles to functional significance. Neurophysiol Clin 33:279–292
33. May A (2008) Chronic pain may change the structure of the brain. Pain 137(1):7–15
34. Treede RD, Lorenz J, Baumgärtner U (2003) Clinical usefulness of laser-evoked potentials. Neurophysiol Clin 33(6):303–314
35. Haanpää M, Attal N, Backonja M, Baron R, Bennett M, Bouhassira D, Cruccu G, Hansson P, Haythornthwaite JA, Iannetti GD, Jensen TS, Kauppila T, Nurmikko TJ, Rice AS, Rowbotham M, Serra J, Sommer C, Smith BH, Treede RD (2011) NeuPSIG guidelines on neuropathic pain assessment. Pain 152(1):14–27
36. Coppola G, Pierelli F, Schoenen J (2009) Habituation and migraine. Neurobiol Learn Mem 92(2):249–259
37. Tampin B, Briffa NK, Slater H (2013) Self-reported sensory descriptors are associated with quantitative sensory testing parameters in patients with cervical radiculopathy, but not in patients with fibromyalgia. Eur J Pain 17(4):621–633
38. Burstein R, Jakubowski M (2010) Managing migraine associated with sensitization. Handb Clin Neurol 97:207–215
39. Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, Broglio L, Granieri E, Lauria G (2008) The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain 131(Pt 7):1912–1925
40. Tavee J, Zhou L (2009) Small fiber neuropathy: a burning problem. Cleve Clin J Med 76(5):297–305
41. Eijkelkamp N, Linley JE, Baker MD, Minett MS, Cregg R, Werdehausen R, Rugiero F, Wood JN (2012) Neurological perspectives on voltage-gated sodium channels. Brain 135(Pt 9):2585–2612
42. Furuta A, Suzuki Y, Hayashi N, Egawa S, Yoshimura N (2012) Transient receptor potential A1 receptor-mediated neural crosstalk and afferent sensitization induced by oxidative stress: implication for the pathogenesis of interstitial cystitis/bladder pain syndrome. Int J Urol 19(5):429–436
43. Klein CJ, Lennon VA, Aston PA, McKeon A, Pittock SJ (2012) Chronic pain as a manifestation of potassium channel-complex autoimmunity. Neurology 11:1136–1144
44. Lauria G, Merkies IS, Faber CG (2012) Small fibre neuropathy. Curr Opin Neurol 25(5):542–549
45. Waxman SG (2005) Sodium channel blockers and axonal protection in neuroinflammatory disease. Brain 128(Pt 1):5–6

Journal : Large 415 | Dispatch : 17-12-2013 | Pages : 12
Article No. : 7211 | ☐ LE | ☐ TYPESET
MS Code : JOON-D-13-01229 | ☑ CP | ☑ DISK

Journal : **415**
Article : **7211**

Springer
the language of science

Author Query Form

Please ensure you fill out your response to the queries raised below and return this form along with your corrections

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	Please check and confirm this mail id 'marina.detommaso@uniba.it' should appear in the publication.	
AQ2	Please confirm the section headings are correctly identified.	
AQ4	Please check and confirm the citation of Table 3 is manually inserted in the sentence of 'Fibromyalgia patients with migraine …… also when compared to other FM groups (Table 3; Fig. 3b)'.	
AQ5	Please check and confirm the inserted citation of Fig. 5 is correct. If not, please suggest an alternative citation. Please note that figures should be cited in sequential order in the text.	
AQ6	Please provide an ethical standard statement.	
AQ3	Please provide a definition for the significance of 'bold' in the Tables 1, 2 and 4.	