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Laser-Evoked Potential Characteristics in Fibromyalgia

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LASER-EVOKED POTENTIAL CHARACTERISTICS IN FIBROMYALGIA

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1 Abstract

1.1 Aims

This research aims at examining the relevance of laser-evoked potentials (LEP) as a diagnostic tool for fibromyalgia syndrome (FMS) and deriving possible pathophysiological mechanisms explaining its neuropathic-like symptoms.

1.2 Methods

Retrospective electrophysiological data of 85 FMS patients were recovered from a large patients' database, collected over a 10-year period, and newly submitted to an unbiased automated analysis. Standard references are obtained from two control groups, matching in gender and age.

1.3 Results

LEP showed significantly increased amplitudes in FMS compared to healthy controls (HCs), whereas latencies remained similar. Habituation to repetitive stimuli was found to be reduced in the FMS sample.

1.4 Conclusions

Reduced habituation of cortical responses to laser stimuli in FMS suggests alterations in the pattern of cortical excitability facilitated by abnormalities in central neurotransmission. Increased LEP responses indicate central sensitization. These findings support the usefulness of LEP to diagnose dysfunction of the nociceptive system in FMS. Both peripheral and central abnormal functioning of the nociceptive system may act as pathophysiological mechanisms of FMS.

1.5 Keywords

Fibromyalgia syndrome - Chronic pain - Peripheral nervous system dysfunction - Central nervous system dysfunction - Small nerve fibres - Aδ fibres - Nociceptive pathways - Laser-evoked potentials - LEP - CO_2 laser - Pain related evoked potentials - N1 - N2 - P2 - habituation - Single-trial analysis - Independent component analysis - Wavelet filtering - Multiple linear regression

2 Introduction

2.1 Fibromyalgia

Prosecuted for many decades, the fibromyalgia syndrome (FMS) finally crawls out of the depths of the pre-Victorian era into today's enlightened age. In 1990, the American College of Rheumatology (ACR) introduced the FMS classification criteria, setting sail towards an increased recognition of this syndrome (1). In recent years, an increasing number of open-minded research groups have shed their light on the unexplained and complex pathophysiology underlying this disabling disorder. Fibromyalgia, with its subjective and heterogeneous symptoms, disputable gold standard, compelling nature for research strategies, and yet omnipresent in society, is hitherto an unmet and unique challenge for the scientific and medical community. In order to better understand the diagnostic approach for FMS described in this manuscript, it seems necessary to briefly recapitulate the current clinical and pathophysiological concepts of this condition.

2.1.1 Epidemiology

From recent studies, the prevalence of FMS appears to be 1 - 5 % in the Western countries (2). Up to 5% of the global population seems to be affected (3). Observed as the second most frequent condition in the rheumatology departments (after osteoarthritis) and a substantial subset of the primary care patient population, FMS is far more common than generally suspected among clinicians (4).

Though an estimated 2 to 3% of the overall patient population fulfils the 1990 ACR classification criteria (1, 5), 75 % of FMS patients remain undiagnosed(4). FMS predominantly affects women, with a male to female ratio between 1:3 and 1:4 (2). Additionally, several studies have demonstrated a strong familial predisposition with an odds ratio of 6 to 7 when a first-degree relative is affected (6).

Furthermore, this condition leaves a deeply embedded financial footprint in our socio-economic context. FMS has a profound impact on the patient's daily life, social interactions and job performance leading to a substantial burden on the individual productivity and quality of life (2-4, 7).

Taking into account the limited efficacy of today’s therapeutic options, FMS represents a clinical challenge that should not be underestimated (5).

2.1.2 Symptoms and signs

The cardinal symptoms of FMS : (1, 2, 4, 8-10)

- Chronic widespread pain (CWP)
  - during ≥ 3months (8)
  - localized in the axial skeleton, in at least four quadrants of the body (upper/lower, right/left) (8)
  - clinical signs of sensitization of the central nervous system (5)
  - generalized increase of pain sensitivity (10)
  - generalized deep pain in muscles and tendons (1)
  - appears to be closely related to neuropathic pain (7)
- (Multifocal) tenderness on palpation :
  - decreased mechanical pressure pain thresholds, particularly over pre-designated tender points (fig. 1)
  - central amplification of pain perception : (4, 5)
    - allodynia : a heightened sensitivity to stimuli that are not perceived as painful in healthy individuals (dynamic tactile -)
    - hyperalgesia : an increased response to painful stimuli compared to responses of healthy individuals (secondary pressure -)

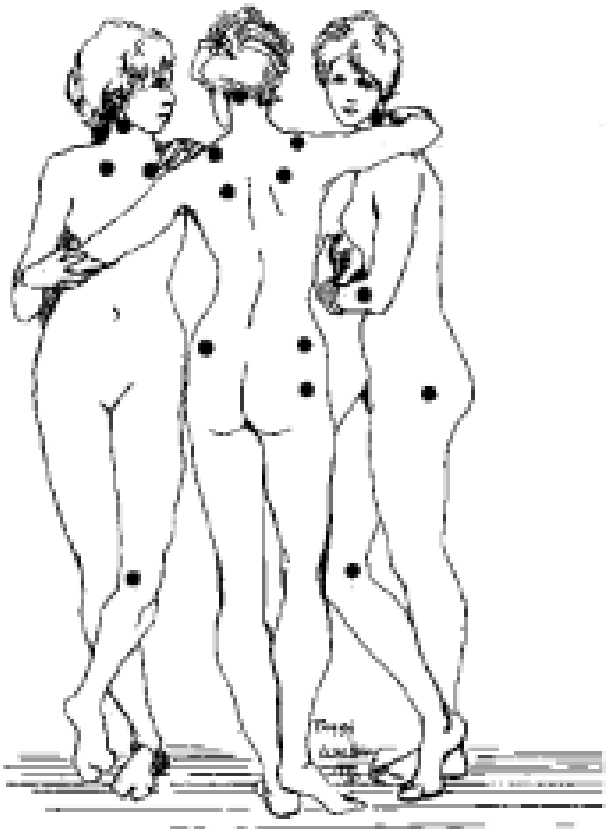


Fig. 1 *Tender point locations following the 1990 classification criteria for FMS ("The Three Graces" after Baron Jean-Baptiste Regnault (1793), Louvre Museum, Paris) [adapted from Wolfe et al (1990) (1)]*

Additional symptoms :

- non-restorative sleep / Unrefreshed awakening
  (sleep disruptions at a variety of stages, with frequent arousals) (5)
- fatigue
- cognitive dysfunction

Other symptoms occasionally described in the literature:

- extent of somatic symptoms (8)
- associated depressive symptoms (7, 9)
- psychological distress (6)

Note : an article by Clauw et al (2011) indicates that the decreased threshold / increased sensitivity exist, for a variety of different sensory stimuli, including heat, cold, auditory and electrical stimuli. (4)

Currently, the main specialized research groups in this field agree on the existence of various co-morbid conditions (5), and describe a heterogeneous constellation - and variability over time of the (predominant) FMS symptoms (and their location). (4, 6)

2.1.3 Pathophysiology

Throughout the last 2 decades extended research have shifted the FMS from a biological blur to a pathophysiological background based on various phenomena as described below. It remains unclear if these phenomena possess a causal relationship, act in a synergic way or - exist concomitantly. Considering the apparent complexity of FMS's pathophysiology, it is of no surprise that this medical condition is particularly difficult to classify amongst more conventional pain syndromes (inflammatory? degenerative? dysfunctional?). Luckily, research into FMS is in constant evolution and the quest to unravel the aetiology of this syndrome elicits an increasing number of protagonists.

2.1.3.1 Decrease of conditioned pain modulation in the central nervous system

The conditioned pain modulation theory describes the decreased capacity of the brain to inhibit and modulate nociceptive input, resulting in a net increase of the nociceptive central response (5).

It comprises a neurochemical imbalance between the neurotransmitters (NT) and/or neurochemicals (and their receptors), which mediate and propagate central nervous system (CNS) signals, in ascending and descending pathways (4, 11).

In healthy controls, peripheral nerves transmit sensory signals (nociceptive) to the spinal cord for transmission through the ascending pathways. Subsequently, the descending pathways send facilitating and inhibiting signals from the brain to the periphery. This balanced process determines the so-called 'volume control setting' on incoming signals, which in turn fine-tunes the pain perception (4).

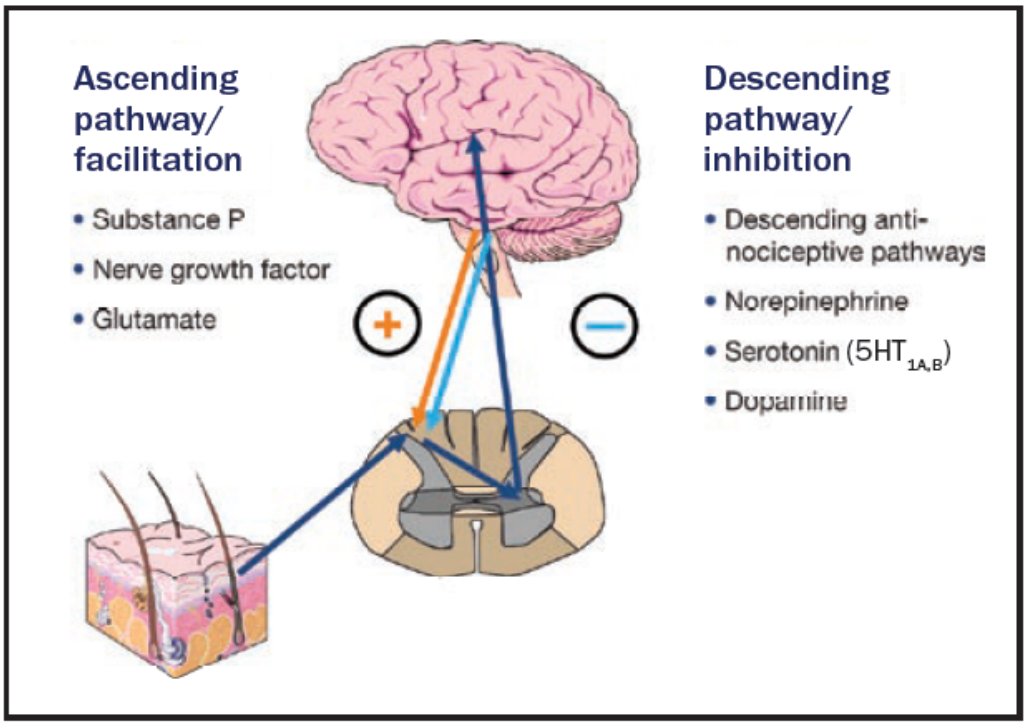


Fig. 2 *Neural pathways and NT influencing pain sensitivity and sensory processing (from Rheum Dis Clin N Am) [adapted from Clauw et al (2011) (4)]*

In FMS it appears that the 'volume control setting' is adjusted towards abnormally high, irrespective of peripheral nociceptive input. An increased processing of the ascending pathways was objectified by elevated levels of specific NT in the cerebrospinal fluid (i.e. serotonin, norepinephrine,...), considered as proclaimers of central excitation. In the descending pathways, an active process of decreased conditioned pain modulation at spinal level was objectified by a decrease in these metabolites (Fig. 2) (4).

The 'volume control setting' decides the lifetime susceptibility for nociceptive - and other sensory signals, resulting in a broad individual variety. It is determined by genetic and environmental factors (11, 12).

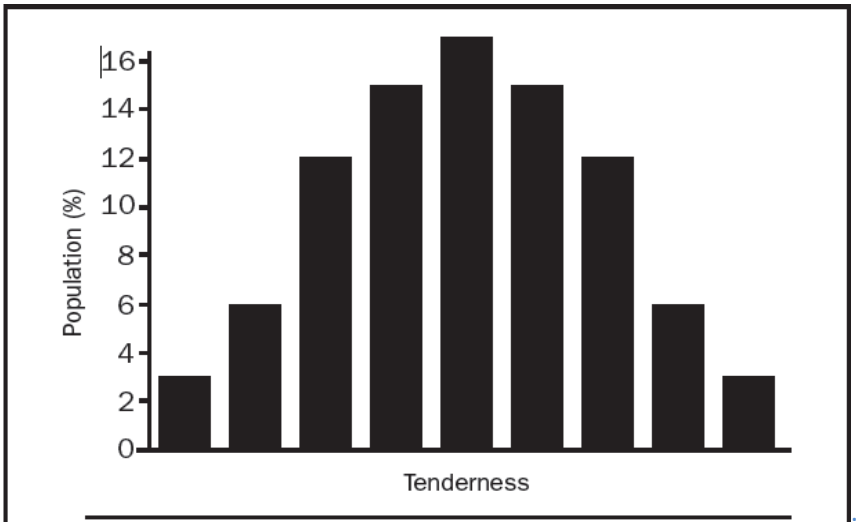


Fig. 3 *Pain sensitivity in the general population.*
[from Rheum Dis Clin N Am) [adapted from Clauw et al (2011) (4)]

The individual susceptibility to pain in the general population displays a normal Gaussian distribution (Fig. 3). The higher an individual is located to the right of this bar chart, the higher the 'volume control setting' and consequently, the more pain one experiences, independently of the level of peripheral nociceptive input. In FMS, the bell-curve is shifted towards the right, resulting in a higher 'volume control setting', a lower pain threshold and therefore a higher pain intensity than healthy controls. This inadequate central neural excitability and particularly deep-tissue impulse pain processing, results in a bio-behavioural model of reactivity to painful (and probably multimodal) stimuli (4, 11, 12).

The following co-existing phenomena in FMS are described in scientific literature:

- FMS patients have high endogenic opioid release, resulting in a diminished opioid receptor availability (4).
- these neuro-processing mechanisms could possibly underlie the observed mood disorders, fatigue and sleep dysfunction (4).
- progressively, the strong CNS component is or becomes mainly independent of the peripheral nociceptive input (4, 9, 11, 12).

2.1.3.2 Amplification of pain signalization in the central nervous system

Central amplification, also called "central sensitization", is a concept introduced by Woolf et al (2011) and applied to FMS by Yunus et al (2007) (13, 14).

Central sensitization is considered to result of an abnormal function of the ascending (i.e. activity dependent increase in synaptic spinal function) and descending pathways (i.e. a lack of saliency), and to be associated with a neurochemical imbalance (glutamate acting on N-methyl-D-aspartate

receptors), resulting in progressive pain wind up (hyperalgesia) after repeatedly painful stimulation, until normal stimuli are perceived as noxious (allodynia). The causal factors for initiating and maintaining the abnormal pain processing remain unknown (4, 6, 13, 15).

The severity of FMS symptom is determined by the individual variety in sensory sensitization, genetical and environmental factors (11, 12).

The modified central neuronal excitability may also be involved in the pathophysiology of other 'dysfunctional' chronic pain syndromes like migraine, temporomandibular joint dysfunction (TMJ), irritable bowel syndrome (IBS), explaining overlapping clinical and biomolecular findings in these clinically heterogeneous pathologies (9).

2.1.3.3 Interaction in pain processing between different areas of the brain

Several brain regions (sensory, affective, cognitive) interact in processing incoming nociceptive stimuli and in generating the ultimate subjective pain experience (4, 5).

Neuroimaging studies have shown increased activity and connectivity between several brain areas in FMS patients, e.g. default mode network, posterior insula, anterior cingulate cortex... as compared to healthy subjects (4, 16, 17). These findings suggest that noxious stimuli create a much larger activation of these specific cortical regions and a lack of reduction in cortical recruitment across repetitive stimuli (9).

Also structural changes to the brain of FMS patients have been observed, such as a loss of gray matter volume in the prefrontal cortex, amygdalae and anterior cingulate cortex (18, 19).

More recently, some authors suggested that the central interaction theory lacks factual basis (2). Abnormal findings on structural and functional neuroimaging studies have been reinterpreted as being nonspecific consequences of chronic pain, rather than causal for the chronic pain condition (2). This does not mean that the excitability of the central nociceptive circuitry is not a possible important clue, which could help unravel the causality of FMS in the future and give this theory the establishment it possibly deserves (4, 9, 13, 20).

2.1.3.4 Small fibre pathology of the peripheral nervous system

After exploring the CNS, research recently has turned again towards the peripheral nervous system (PNS) (2, 4, 10, 21-23). Previous studies had excluded that muscular abnormalities would be causal for FMS (5, 8). Nowadays, several search groups are casting new light on the involvement of the PNS as a main factor contributing to the central phenomena described above. Koroschetz et al (2011) showed a common pathophysiological mechanism between neuropathic pain disorders and FMS (24, 25). Uceyler et al (2013), followed by Oaklander et al (2013) and deTomasso et al (2014), showed pathological and physiological evidence of small fibre impairment in FMS. Each of these studies showed structural and/or functional small fibre disease evidenced by a reduction of intra-epidermal

nerve fibre density and decreased regeneration of unmyelinated C nerve fibres and their associated Schwann cells (2, 7, 17).

Oaklander et al (2013) diagnosed both large-fibre polyneuropathy and small fibre neuropathy (SFN) (with mainly dysimmune aetiology) in resp. 33% and 41% of their FMS-labelled patients, based on electro-diagnostic testing, skin biopsy and autonomic function testing (AFT) (2). It could be considered that either (a) FMS were misdiagnosed and were in fact polyneuropathy patients, or that (b) many FMS patients present with associated neurological symptoms such as polyneuropathy.

Meanwhile, microneurography studies have concluded that the electrophysiological features of the small fibre involvement in FMS patients significantly differ from abnormalities found in SFN patients (without FMS) (25). This strengthens the hypothesis that both conditions emerge from different pathophysiological backgrounds.

The CWP symptoms in FMS patients are neuropathic-like symptoms (NLS). NLS are defined as deafferentation pain symptoms, resulting from damage to the nervous system, and they include paresthesias, burning -, tingling - and prickling pain, spontaneous -or abnormally provoked pain sensations (60% of these patients have allodynia and/or hyperalgesia) (24, 26).

The fact these sensory FMS symptoms (numbness, prickling, burning, allodynia, hypersensitivity to thermal - and mechanical stimuli) are NLS, gives opportunity to some research groups, rather premature, to propose an allocation of FMS to the SFN (2, 24, 25). This proposition finds foundations in the fact that (a) Koroschetz et al (2011), using symptoms questionnaires, observed an overlap of sensory descriptors in 20–35% of patients with FMS and SFN (24, 25), (b) a previous study reported dysesthesic sensory disturbances in up to 84% of FMS patients, sometimes in a stocking–glove distribution typical for peripheral neuropathy (24, 27), (c) SFN and FMS can have similar localizations (SFN: occasionally generalized) distal-proximal (if advanced SFN) gradient, and, (d) both disorders can have disturbed efferent effects of somatic and autonomic small-fibre s on internal organs, blood vessels, and sweat glands (2).

Several, non-SFN related, mechanisms could be hypothesized to account for neuropathic-like symptoms in FMS:

1. FMS patients could have a peripheral filtering deficit in their skin-, muscle- and joint afferents. Healthy small fibres posses a filter function, which allows them to conduct only a small fraction of all elicited action potentials. In FMS, by losing this barrier function, the small fibres would conduct unselectively the majority of all afferent nociceptive stimuli volleys (7).
2. In FMS patients, a reduction of the mechano-sensitive C-fibres , conducting pleasant touch, could lead to a relative increase in pain perception (7).
3. Neuropathic-like pain can be elicited by sensitization of the nerve endings in the skin from inflammatory mediators (7).

2.1.3.5 Conclusion

To the extent of our knowledge, FMS is nowadays considered as a chronic disorder of the CNS pain processing, with associated PNS impairment. The current state of research does not allow concluding on the causal relationship or balance between CNS and PNS abnormalities. Unfortunately, few research methods yield objective measurements of nervous system dysfunction. Therefore the identification of causal or risk factors, the development of an indisputable gold standard for diagnosis and the search for disease-modifying treatments remain a great challenge and as great opportunity for future research.

2.1.4 Differential diagnosis

2.1.4.1 Small fibre polyneuropathy

SFN belongs to a delineated subgroup of the sensory neuropathies. It is defined as a disease caused by the dysfunction and degeneration of peripheral small fibre neurons, more specifically the unmyelinated C - and thinly myelinated Aδ fibres (gangliono- and neuronopathy). It can be accompanied by disturbed somatic and autonomic efferent effects. The main manifestation is superficial burning pain and dysesthesia with a distal-proximal gradient (2). Proximal spread is possible, but only found in rare advanced cases (7). Fatigue and unrefreshing sleep are uncommon in SFN (28). A substantial subset of the SFN patients displays additional cardiovascular, gastrointestinal, microvascular and sweating complaints due to disturbance of autonomic small fibres. Common aetiologies of SFN are diabetes, haematological malignancies, autoimmune conditions, infections, toxins (including medications) and genetic mutation (2).

Several authors (Koroschetz et al (2011), Oaklander et al (2013), Uceyler et al (2013), DeTomasso et al (2014)) have established a link between FMS and SFN (2, 7, 17, 25). Likewise, clinicians report that a substantial number of patients, considered to have FMS, are subsequently diagnosed with SFN. Oaklander et al (2013) hypothesises that some patients labelled as FMS, have unrecognized SFN, basing this assumption on reduced intra-epidermal nerve fibre density and altered function of the autonomic nervous system (2). Uceyler et al (2013) were more careful interpreting their data: these authors concluded that every case of small fibre pathology is not automatically SFN (7). According to recent diagnostic criteria, SFN is characterized by a typical clinical presentation, which is not found in many FMS patients (7, 28, 29). Finally, microneurography studies indicate that the electrophysiological features of the small calibre nerve fibres in FMS patients differ significantly from those of SFN patients (7) (see section 2.2.8). This strengthens the notion that both conditions emerge from different pathophysiological backgrounds.

Given the fundamentally different pathophysiology of FMS and SFN (see above), it is impossible to consider that FMS is a variety of SFN. If FMS patients present with abnormalities compatible with SFN, this probably should be interpreted as a co-existence of both diseases or as a misdiagnosis of FMS (presumably a subset of patients with sensory and autonomic symptoms).

2.1.4.2 Persistent somatoform pain disorder according to DSM-IV

In some countries, the idea prevails that FMS is a non-existing clinical entity and thus should be ignored as a clinical diagnosis. This ideological situation may be regarded as opposing a camp of 'believers' to 'non-believers'. As a direct consequence in these countries, FMS patients are diagnosed either with a persistent somatoform pain disorder (PSPD) according to the DSM-IV (300.81) diagnostic criteria. In fact, PSPD and FMS are separate entities. A recent study by Uceyler et al (2013) showed a clear distinction between FMS and PSPD, and pointed out that FMS may be associated with depressive symptoms without meeting the diagnostic criteria for major depression or for PSPD. In conclusion, FMS is not, as believed by many for decades, a 'merely' psychological disorder (though it clearly affects the CNS). Besides, psychogenic pain or somatisation disorders do not exclude a diagnosis of FMS, according to the 1990 ACR criteria for FMS. Contradictory, according to the 2010 ACR criteria for FMS, the diagnosis of any other disorder does exclude a diagnosis of FMS, but, to this day, there are still many opponents to that recommendation (1, 6, 7).

2.1.4.3 Central sensitivity syndromes / Chronic dysfunctional pain syndromes

Distinct chronic pain syndromes (CPS) have been studied to explain the clinical and pathophysiological overlap between some ostensibly different disorders. This group of CPS have been classified by some as nonorganic, or as central sensitivity syndromes (CSS). CPS have common features, described by Woolf et al (2011), as their high prevalence and maladaptive forms of pain plasticity. Clinical cohort studies in chronic pain populations have shown changes in pain sensitivity compatible with central sensitization phenomena (13).

FMS is considered as a prototypical example of CSS. Phillips et al (2011) described following clinical entities (Fig.4) as currently considered parts of the spectrum of CSS (6) :

- FMS
- Chronic fatigue syndrome (CFS)
- IBS and other functional GI disorders
- Temporomandibular joint disorder (TMJD)
- Restless Leg Syndrome (RLS) and Periodic Limb Movements in Sleep (PLMS)
- Idiopathic Low Back Pain (LBP)
- Multiple Chemical Sensitivity (MCS)
- Primary Dysmenorrhea
- Headache (tension > migraine, mixed)
- Interstitial Cystitis/Chronic Prostatitis/Painful Bladder Syndrome/prostadynia
- Chronic Pelvic Pain, Endometriosis
- Myofascial Pain Syndrome / Regional Soft Tissue Pain Syndrome

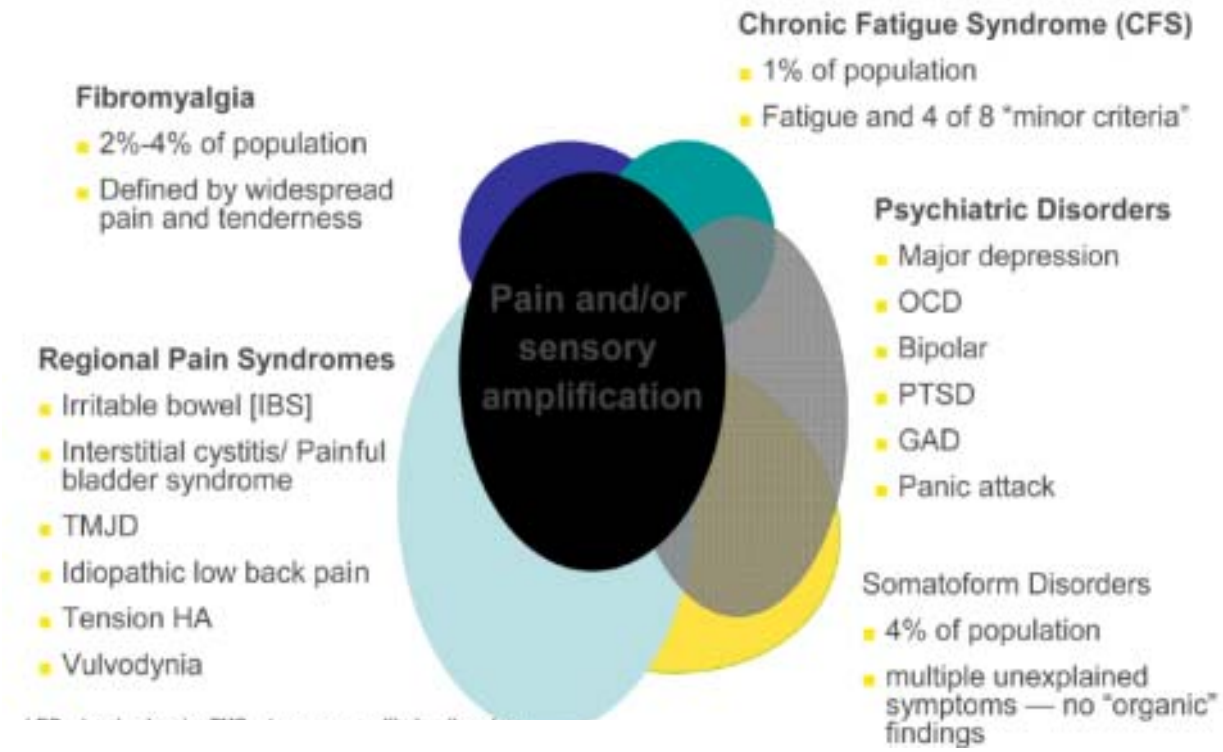


Fig. 4 *Sensory amplification overlap between systemic syndromes (from Neuroimmunomodulation) [adapted from Clauw et al (1997)/Phillips et al (2011) (4, 6)]*

Some questions remain unanswered e.g. Is there a higher inherited risk for developing CSS in some specific individuals?

2.1.4.4 Conclusion

Do some clinicians label 'annoying' patients as FMS , without further examination or questioning for atypical manifestations of some diseases like SFN? Do we sometimes fail in recognizing FMS due to cultural beliefs or different specialty-related visions? As long as the pathophysiology is not completely unravelled or an objective, irrefutable diagnostic tool can be imbedded in the clinical work-up of FMS patients, these questions will remain unanswered for the time being.

The 2010 ACR criteria (8) clearly state that the diagnosis of FMS is excluded if any other clinical disorder may explain the patient's symptoms. Many prominent research groups disagree with this 'exclusion' concept and support the opposite (based on the 1990 ACR criteria (1) :
they consider the diagnosis of FMS non-exclusive of other pathologies with similar symptoms. Thus, FMS could be a secondary, independent, causal, co-existing, ... disorder. These different perspectives on 'primary' (= exclusive) and 'secondary' (= co-existing morbidity) FMS does not preclude the need for an exhaustive additional work-up (blood tests, imaging, etc...) before considering this diagnosis.

2.1.5 Established methods for diagnosing fibromyalgia

The central problem in diagnosis FMS is the lack of an absolute (gold) standard (8). It should be a top priority for the scientific community to find objective physical or laboratory features, or well-characterized pathologic findings for this syndrome. Unfortunately, FMS remains undiagnosed in an estimated 75% of all affected individuals, leading to inadequate management of their condition (4).

The current clinical approach (Table 1) in diagnosing FMS is based on the 2010 ACR classification criteria; this includes a widespread pain index (WPI) and symptom severity scale (SS) (8). The de facto gold standard for FMS currently accepted is the WPI and the tender point count as described in the 1990 ACR classification criteria. They should be examined at the predetermined locations with a pressure of at least 4 N (i.e. 4 kg/cm²) (4). Tender points are considered by some clinicians as the key point feature to differentiate FMS from other disorders. Other clinicians consider tender points a very subjective parameter subject to considerable variability. An appropriate physical examination (including tender point sites) remains highly recommended for all (FMS) patients (8).

The 1990 ACR criteria performed well in specialty clinics (1), but they haven't been widely used in primary care. Over time, it became clear that the tender point count was rarely performed, and if so, it was performed incorrectly. Many physicians lacked the training to examine them properly or simply refused to do so (8). The 2010 ACR classification criteria are based on a questionnaire, without any form of clinical examination. The development of these criteria was not aimed at routine clinical use (8). Thus, the usefulness of the 1990 and 2010 ACR criteria is dependent on the clinical setting.

Table 1 *Sensitivity and specificity (in %) of the 1990 - and 2010 ACR criteria for diagnosing FMS [Based on Wolfe et al 1990 and 2010) (1, 8)]*

	1990	2010
Sensitivity	88.4 %	88.1 %
Specificity	81.1 %	93.8 %

The FMS diagnosis is a symptom-based diagnosis. The importance of the symptoms has not been considered in the 1990 criteria, resulting in approximately 25% diagnosed FMS patients by their physicians, who did not satisfy the first ACR classification criteria. Besides, FMS experts believed that the tender point examination could mistakenly link this disorder to peripheral muscle anomalies. The 2010 criteria represent an alternative method of diagnosis, and do not replace the criteria of 1990.

2.1.5.1 The ACR 1990 FMS classification criteria

Patients are diagnosed with FMS if both following criteria (1) are satisfied:

1. History of CWP

Defined by pain for at least 3 months in all the following areas combined: the left - and right side of the body, pain above and below the waist, and, axial skeletal pain (cervical spine, anterior chest, low back or thoracic spine).

2. Pain in 11 of the 18 tender point sites on digital palpation with an approximate force of 4 N.

Tender point sites (Fig. 1):

- Occiput: bilateral, at the suboccipital muscle insertions
- Low cervical: bilateral, at the anterior aspects of the intertransverse spaces at C5-C7
- Trapezius: bilateral, at the midpoint of the upper border
- Supraspinatus: bilateral, at origins, above the scapula spine near the medial border
- Second rib: bilateral, at the second costochondral junctions, just lateral to the junctions on upper surfaces
- Later epicondyle: bilateral, 2cm distal to the epicondyles
- Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle
- Greater trochanter: bilateral, posterior to the trochanteric prominence
- Knee: bilateral, at the medial fat pad proximal to the joint line

A tender point is considered positive if the subject states palpation was painful. Thus 'tender' points may in fact be non-painful.

These criteria make no exclusion of the presence of concomitant radiographic, clinical or laboratory abnormalities; the presence of an additional clinical disorder does not exclude the diagnosis of FMS (1, 8, 30).

2.1.5.2 The ACR 2010 FMS diagnostic criteria

A patient satisfies diagnostic criteria for FMS (8) if the following 3 conditions are met:

1. Widespread pain index (WPI) ≥7 and SS scale ≥5
OR WPI between 3-6 and SS scale ≥9

2. Symptoms have been present at a similar level for at least 3 months

3. The patient does not have a disorder that would otherwise explain the pain.

Questionnaire (8) :

* WPI: note the number of areas in which the patient has had pain over the last week: shoulder girdle (left/right), upper and lower arm (left/right), buttock or trochanter (left/right),upper and lower leg (left/right), jaw (left/right),chest, abdomen, upper and lower back and/or neck.

* SS scale score: is the sum of the severity of the 3 FMS symptoms (Fatigue, waking unrefreshed, cognitive symptoms) plus of somatic symptoms in general, over the past week using a scale between 0-3 (no problem>severe).
Somatic symptoms that might be considered: muscle pain/weakness, fatigue/tiredness, thinking/remembering problem, depression, nervousness, seizures, headache, insomnia, numbness/tingling, itching, rash, hives/welts, sun sensitivity, hair loss, easy bruising, blurred vision, hearing difficulties, ringing in the ears, dizziness, loss/change in taste, dry eyes, dry mouth, oral ulcers, Raynaud's phenomenon, frequent urination, painful urination, bladder spasms, IBS, nausea/vomiting, diarrhea/constipation, upper abdominal pain, abdominal pain/cramps, loss of appetite, fever, heartburn, chest pain, shortness of breath, wheezing ...

(Note: the SS scale can be used at any time regardless of the diagnostic status.)

2.1.6 Therapeutic management of fibromyalgia

Current treatment approaches are often insufficiently effective. Due to chronic drug use, many patients experience side effects, organ dysfunction and/or interaction with other medications. Nevertheless, the multimodal treatment strategy described below, combining pharmacological as well as non-pharmacological therapy, is supported by the highest level of evidence currently available. In all cases, it is recommended to practice a highly personalized approach, and to introduce therapeutic interventions gradually and with great care. The complexity of this syndrome calls for a highly innovative approach on the part of both clinicians and researchers. It is very likely that FMS management in a decade may prove to be very different than the one currently applied (2, 5).

2.1.6.1 Pharmacological therapy

Some patients are distinctly unwilling to accept pharmacological treatment. It is worth mentioning that many mild cases of FMS may be successfully handled with non-pharmacological treatment (5).

Centrally acting pharmacological agents are most effective in patients with CSS (13). Based on extensive review of the current scientific pharmacological literature, the only drugs approved by the American FDA for the treatment of FMS are pregabaline (<α2δ ligands) and duloxetine (<SNRIs) (5).

Combined norepinephrine dopamine reuptake inhibitors, substance P antagonists, and opioid antagonism are intriguing possibilities for the future. Canabinoid agonists hold promise in the treatment of FMS, but current evidence is incomplete. Sodium-oxybate, a unique sleep promoting medication is also under investigation (5).

Opioids, NSAIDs, corticosteroids, benzodiazepine and non-benzodiazepine hypnotics can be found among many others in the extensive pool of *not* evidence-based therapeutic options for FMS (5).

At all times, emphasis should be given to individualized analgesia in every chronic pain patient.

2.1.6.2 Rehabilitation of fibromyalgic patients

The recent guidelines for the management of FMS emphasize the importance of implementing non-pharmacological therapeutic interventions. These are in many ways more desirable than relying solely on medications. There are many options, including electrotherapy, biofeedback, relaxation, cognitive behavioural therapy, mindfulness, educational interventions, hydrotherapy, strengthening exercises, endurance training, etc. (5).

2.1.7 Conclusion

Although the last decades awareness of FMS have improved, this syndrome remains undiagnosed in as many as 3/4 of FMS patients. To the extent of our knowledge, FMS is now described as a chronic pain disorder of the CNS pain processing, with associated PNS impairment. The current state of research does not allow concluding on the causal relationship or balance between CNS and PNS abnormalities. Unfortunately, few research methods yield objective measurements of nervous system dysfunction. Therefore the identification of causal or risk factors, the development of an indisputable gold standard for diagnosis and the search for disease-modifying treatments remain a great challenge as well as a great opportunity for future research.

2.2 Assessment of the somatosensory system

A background of spontaneous brain electrical activity can be detected continuously by electroencephalography (EEG). In contrast, the presentation of a stimulus (e.g. visual, auditory, electrical, laser,...) elicits an electrical response in the CNS that is time-locked to the stimulus and named evoked potential (EP) (15).

2.2.1 Structure and function of the somatosensory system

The multimodal somatosensory system consists of two pathways: the tactile-proprioceptive - and the thermo-nociceptive pathway (15).

The first, but alas not the second, can be investigated electrophysiologically by conventional somatosensory evoked potentials (SEP). This reflects only the function of the large fibres, dorsal columns, medial lemniscus and their thalamo-cortical projections (16).

In the second half of the somatosensory system, thermo-nociceptive signals are conveyed by small C- and Aδ-afferents, to the ventrolateral spinothalamic tract with thalamo-cortical projections: i.e. the ventro-postero-lateral nucleus (VPL) to the primary somatosensory cortex (SI), the ventro-postero-inferior nucleus (VPI) to the secondary somatosensory cortex (SII) and the posterior ventro-medial nucleus (VMpo) to the dorsal insula. The first two receive also projections from the tactile-proprioceptive pathway (Fig. 5) (15).

Widely agreed the most reliable tool, in other words the gold standard, for investigating the Aδ-fibre pathway is laser evoked potentials (LEP), i.e. evoked potentials elicited with a laser beam stimulus (16, 31, 32).

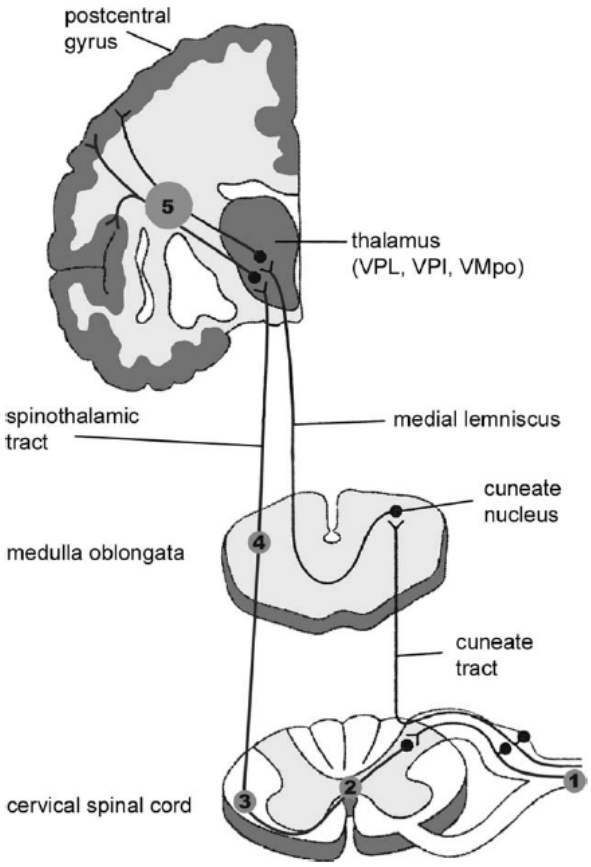


Fig. 5 *Somatosensory pathways and lesion sites assessed by LEP*
[Adapted from Treede et al (2003) (15)]

Pain is currently defined by the International Association for the Study of Pain as 'an unpleasant sensorial and emotional experience related to present or potential tissue damage, or described in such terms' (33). While different pain classifications exist, pain is most commonly categorized as : nociceptive, inflammatory, neuropathic or dysfunctional (20). Other classifications are based on the localisation and nature of tissue lesions (Table 2).

Table 2 *Mechanistic characterization of pain. Any intra-individual combinations may be present*
[Based on an article by Phillips et al (2011) (6)]

	PERIPHERAL nociceptive	NEUROPATHIC neuropathic	CENTRAL dysfunctional
Aetiology	Inflammation / mechanical damage in all tissues	Damage / entrapment of peripheral nerves	Central disturbance in pain processing
Responsive to	NSAIDs Opioids	NSAIDs Opioids Na channel blockers TCA's Neuroactive compounds	 TCA's Neuroactive compounds
Behavioural factors	minor		prominent

Nociception, meaning the activation of the nociceptive nervous system, must be distinguished from the perception of pain as a subjective experience. In the assessment of pain, physicians and researchers aim to distinguish between nociception and pain by using (semi-)objective assessments placing the subject under conditions as standardized as possible (20).

Table 3 *Assessment methods by sensation for nerve function of the large thick - (Aβ), small thinly - (Aδ) and small non - myelinated (C) fibres [Adapted from Cruccu et al (2010) (32)]*

Fibre	Sensation	Clinical	Psychophysics	Electrophysiology
Aβ	Touch	Piece of cotton wool	Von Frey filaments	NCS SEP
	Vibration	Tuning fork (128 Hz)	Vibrameter	-
Aδ	Pinprick, Sharp pain	Wooden cocktail stick	Weighted needles	Nociceptive reflexes, LEP
	Cold	Thermorollers	Thermotest	-
C	Warmth	Thermorollers	Thermotest	LEP
	Burning (+ itch and light touch)	-	Thermotest	LEP

The function of the somatosensory system can be examined, as summarized in Table 3, with eliciting corresponding perceptual experiences, a quantification of sensory deficits through the use of specific tools, and psychophysiological - and electrophysiological testing (32).

Only a few external stimuli activate the nociceptive system in a selective and specific way, in contrast with contact thermodes in Quantitative Sensory Testing (QST) or in Contact-Heat Evoked Potentials, and with strong thermal stimuli applied by a thermotest (may simultaneously elicit a pain sensation and activate tactile non-nociceptive afferents through thermode skin contact) (16, 20). This makes studying the activation and transmission of nociceptive stimuli within the somatosensory system a true challenge (16).

2.2.2 Somatosensory evoked potentials

SEP reflects in its early components the small generalized changes in tactile non-painful sensitivity. This assessment is low sensitive to attention and other task-related variables (15).

Disadvantage:

SEP results only reflect the function of the large fibres, dorsal columns, medial lemniscus and their thalamo-cortical projections, mediating sensations like touch and vibration, but not the somatosensory pathway as a whole. In patients who present with a dissociated sensory loss of pain and temperature -, but preserved tactile and proprioceptive sensitivity, standard SEP are therefore of limited value (15). The electrical threshold to activate nerve fibres is lower in myelinated fibres than in non-myelinated fibres. Myelinated fibres have faster conduction velocities. Therefore, the brain response elicited with electrical stimulation will always contain the responses of large fast-conducting fibres , whereas small slow-conducting fibres will not be activated at low electrical intensity; and if activated at high electrical intensity, large fibre responses wipe out the subsequent slow fibre response (16).

2.2.3 Nerve conduction studies

Nerve conduction (velocity) studies (NCS) are the most appropriate method in clinical routine to study peripheral large sensory nerve fibre function (Aβ) (2, 16, 32).

Disadvantage:

- In patients with distal sensory neuropathy, the test values may be normal (16).
- SFN (dysfunction of the slowest conducting nerves) is not detected, because these studies only reflect the fastest conducting nerves (i.e. the heavily myelinated fibres) as explained above (16).
- Even large fibre neuropathy can remain undetected distally, due to the fact that routine NCS usually does not include the sensory nerves below the ankle (16).

2.2.4 Nociceptive withdrawal reflexes

Nociceptive withdrawal or flexion reflexes can be evoked in order to examine the nociceptive afferent pathways. They are seldom used in clinical routine or in clinical human research. (16)

2.2.5 Punch skin biopsy

This clinically relevant method allows, after immunolabelling, quantification of the density of intra-epidermal small nerve fibres and late-stage axonal degeneration. It is an objective diagnostic test for SFN (2, 7, 24).

Disadvantages:

- Only the relative (to another diagnostic method) sensitivity and specificity of this tool is known, due to the lack of a gold standard for FMS (2).
- With this method, it is impossible to make a distinction between afferent and efferent nerves, nor C- and Aδ-fibres. Considering intra-epidermal nerves are mostly C-fibres and the peripheral impairment in FMS is probably situated mainly at the Aδ nerves, it is not the method of choice in clinical or research setting regarding FMS. This may also explain why the intra-epidermal nerve fibre density does not correlate with the pain intensity in FMS patients (34, 35).

Results in FMS research:

- Uceyler et al (2013), followed by 3 other research groups, (2, 7, 17, 24) quantitatively showed a reduction in the intra-epidermale innervations and regeneration of the non- (C) and thinly- (Aδ) myelinated nerve fibres and associated Schwann cells. Though, these skin biopsies with aberrant morphology were not a conclusive explanation for the neuropathic-like pain in FMS (as clarified in section 2.1.3.4).
- Giannoccaro et al (2013) and Oaklander et al (2013) considered skin biopsy in the diagnostic work-up of FMS to search for SFN (2, 24).

2.2.6 Quantitative sensory testing

Hatem et al (2010) described QST as follows: it refers to methods intended to quantify perception of different submodalities in the somatosensory system. QST is particularly useful to describe somatosensory deficits, i.e., an abnormal elevation of sensory perception and/or pain thresholds. It is also useful to quantify mechanical and thermal hypersensitivity, i.e., allodynia and hyperalgesia. There is no consensus regarding a specific algorithm for the assessment of thermal or mechanical allodynia and hyperalgesia. Thermal QST has been used for the diagnosis and follow-up of small fibre neuropathies (Aδ and C fibres) that cannot be assessed by standard nerve conduction. Vibration thresholds are useful to assess large fibre neuropathies such as chemotherapy-induced neuropathies. QST changes are not only found in lesions of the nervous system but also in pain conditions due to musculoskeletal or visceral disease for example. In these non-neuropathic pain states QST abnormal responses may be due to central sensitization phenomena (16).

Disadvantages:

- The extent of the stimulus is mainly controlled by the patient's subjectivity, making this only a semi-objective method concerning FMS diagnosis (depending on perceptual and decision-making factors) (2, 20).
- Only the relative (to another diagnostic method) sensitivity and specificity for this tool is known, due to the absence of a gold standard for SFN, FMS, and other diseases affecting the thermonociceptive system (2).

Results in FMS research:

- QST studies in FMS yield variable results due to differences in methodology, reference values and investigated body regions. Conflicting results are observed on thermal perception and -pain thresholds. Only 2 studies tested QST at the dorsum of the foot and found a trend of increased perception thresholds for heat and cold, indicating Aδ- and C-fibre dysfunction (7).
- With QST, an increase in mechanical detection thresholds was observed in FMS. This supposedly could be explained by a reduction of the number or function of mechanosensitive C-fibres (7).
- FMS patients have reduced habituation to painful heat stimuli (9).

2.2.7 Microneurography

This assessment allows recording of action potentials in single fibres of peripheral nerves in awake subjects in an experimental setting (16).

Results of FMS research:

Microneurographic studies have unequivocally shown a distinction between the electrophysiological properties of small-calibre nerve fibres of patients with FMS and those with SFN (16, 28).

2.2.8 Laser Evoked Potentials

2.2.8.1 Evoked-potentials: mechanism of action

LEP are elicited by a radiant heat source in far infrared, which creates a sufficient heat output (>1.000°C) in low-intensity pulses, creating a sharp rise of the skin surface temperature (within the first 100µm skin depth with a CO_2 laser) (16, 20, 31, 36-38). This temperature increase evokes synchronous discharges in the free nerve endings (Aδ-afferents) in only the superficial hairy skin layers (7, 36). The C-fibres can be stimulated by heat conduction (31). The Aβ-afferents are located to deep (> 100µm of the skin surface), and have few thermo-nociceptors. Therefore, these afferents will never be stimulated by a laser beam.

The activation of the Aδ- and C-nociceptors occurs in a substantially concurrent manner, enabling the recording of LEP, and, provoking a double painful sensation (pin-prick -, followed by diffuse burning) due to different conduction velocities of Aδ- and C-fibres (Table 4) (15, 20).

Laser-evoked brain responses reflect the combined activities of the following cortical regions, as described in numerous source localization analysis studies (39, 40) :

- SII, bilaterally
    - activation reflected mainly by the N1 wave
    - processing of nociceptive information and of innocuous stimuli derived from large receptive fields
- Anterior cingulated cortex (ACG)
    - elaborates the attentive and emotional components of pain
- (Posterior) insular regions, bilaterally
    - chiefly contributory
    - elaborates the attentive and emotional components of pain
- SI, contralaterally
    - chiefly contributory
    - processes fine tactile discrimination
- Limbic areas
    - activation related to the unpleasantness of the LEP stimulus
    - this is the motivational - affective system

With double pulse laser stimuli, Mouraux et al (2004) describe reactivation of these neural populations, generating the N2-P2 component of the LEP, as early as 280 ms after the first activation (41).

Table 4 *Differences between large and small fibres pathologies (20)*

Sensory afferents	**Fibre diameter**	**Myeline sheath**	**Conduction speed**	**LEP wave**	**Pain sensation**	**Note**
Aβ	Large	Heavily	Very fast	/	Tactile	Investigative method of choice: sensory NCS
Aδ	Small	Thin	Fast (10m/s)	Late	Pinprick	Exclusive origin for the N2-P2 peak
C	Small	No	Slow (<1m/s)	Ultra-late	Burning	Only recorded in absence of Aδ co-activation

Few external stimuli have the ability to activate nociceptors selectively, - to assess their conduction speed within the somatosensory pathways, and - to study the neural activity related. Laser stimuli have the advantage of stimulating selectively skin nociceptors without any tactile stimulation (in contrast with contact thermodes in QST or in Contact-Heat Evoked Potentials) (16, 20).

LEP is acknowledged by the European Federation of Neurological Societies as the most reliable laboratory tool available for exploring the (functional state of) the nociceptive, somatosensory system (15, 32, 42). LEP are used in the diagnostic approach of patients with CNS and PNS lesion or dysfunction in specialized algology or electrophysiology laboratories, mainly in Europe. Some research facilities in the US, Japan and China also have access to this technology.

Advantages of LEP:

1. LEP is a non-invasive neurophysiological method for assessing the thermonociceptive system (16, 35, 43).

2. Electrophysiological investigations (such as LEP) are preferred above psychophysical studies in terms of objectivity, because the results of the latter are dependent on the collaboration and attitude of the subject (and thus more subjective) (16).

3. This method does not reflect the intensity of pain, but the functional state (and the degree of impairment) of the nociceptive pathways related to the neural processing of pain perception. Therefore, LEP and subjective pain perception should be dissociated when interpreting results (15, 44).

4. Psychiatric patients with altered pain perception will have normal LEP, due to the fact their nociceptive pathways are intact (6, 15, 16).

5. LEP are particularly interesting to document patients with a dissociated tactile (large : Aβ) and thermonociceptive (small : Aδ-C) perception. (Table 4) Due to a lack of skin contact and a high current density only above the dermal-epidermal junction (superficial), there is in the LEP set-up never an activation of the Aβ-/tactile afferents. Accordingly, LEP only reflect the state of the slowest-conducting fibres sensitive to thermal stimulation (15, 16, 20, 45, 46).

Until now, no information is available on the likelihood of obtaining false-positive or false-negative results with LEP in the context of FMS. However, there are studies describing the specificity and sensitivity of LEP for the diagnosis of SFN, radicular neuropathy, and others (16, 35).

2.2.8.2 Normal evoked-potential values in healthy subjects

LEP were first introduced by Carmon et al in 1976 (47). From that moment on, the quest to find robust normative LEP data was launched. Although many considerable studies have been published on this subject, we prefer to base our statements on the normative values proposed by Truini et al (2005) (Table 5) (31). In this paper, a large sample of normal subjects in a extensive age range are described as well as the effects of some important clinical variables (such as body height, age, gender), as discussed in section 2.2.8.4 (31). Of course, these values depend on the technical aspects of the recording session (signal-to-noise ratio, number of trials, number of sites examined, off-line manipulations of the data) and on the characteristics belonging to the investigator (decision criteria to recognize LEP, experience) (31). These factors could explain the diversity among normative values in current literature.

LEP deflections are usually characterized based on their morphology, scalp topography, polarity, latency relative to stimulus onset and amplitude (peak-to-baseline) (Table 5). The terminology of these terms will also be illustrated in section 2.2.8.4.

Table 5 *Topography, sensory afferents for conduction and normal limits for main LEP after stimulation at hand (H) or foot (F) side for clinical applications*
[Values adapted from Truini et al (2005) (31)]

LEP waves :	**N1**	**N2**	**P2**	**P3a**	**P3b**
properties (Units) :					
Latency (H) (in ms)		236 ± 18	315.4 ± 23.1		
Amplitude (H) (in µV)		18.3 ± 8.5			
Latency (F) (in ms)		275.4 ± 16.7	361 ± 26.3		
Amplitude (F) (in µV)		16 ± 5.5			
Sensory afferent	(Early)	Aδ (Late)	Aδ (Late)		C (Ultra-Late)
Culmination topography	Contralateral midtemporal	Vertex (Cz)	Vertex (Cz)	Fronto-central	Parietal (Pz)
Polarity	negative	negative	positive	positive	positive
Reference	Frontal (Fz)	Earlobes (A)	Earlobes (A)	Earlobes (A)	Earlobes (A)

Truini et al (2005) determined perceptive thresholds (PTh) normal values, whereby the PTh was defined as the lowest intensity in which the healthy subjects perceived at least 50% of the painful (pin-prick) stimuli, thus the threshold for Aδ activation by means of laser (31).

After hand stimulation: 5.7 ± 2.6 mJ/mm²
After foot stimulation: 7.3 ± 4.4 mJ/mm²

Of note, the normative values for the N1 deflection are considered a controversial subject. Treede et al (2003) explained that the N1 wave is generated by neural activities of smaller magnitude than those underlying the N2 and P2 waves (15). Furthermore, the temporal electrodes are often contaminated by artefacts related to the activity of the temporalis muscle (39). Finally, Hu et al (2010) showed that the N1 and N2 waves overlap in time and space with opposite polarities (39). These three factors may contribute to the difficulty to visually recognize the N1 peak of LEP and explain why an automated single trial analysis of the ERP waves may provide an alternative solution for finding the N1 waveform.

2.2.8.3 Aberrant evoked-potentials in fibromyalgia patients

LEP are considered pathological when one or several of the following abnormal findings are recorded: the absence of a peak, a prolonged latency, a diminished amplitude, and/or a treacherous scalp topography (15, 16).

Gibson et al (1994) was the first to explore LEP in FMS patients. Only a few research groups, namely Lorenz et al (1996 and 1998), Granot et al (2001), Garcia-Larrea et al (2002), Valeriani (2003) and DeTomasso (2011 and 2014), have followed his example by investigating if LEP would provide a reliable diagnostic tool for FMS (32). To our knowledge, only these 8 studies, all conducted between 1994 and 2014, were dedicated to examining LEP in FMS.
(9, 10, 17, 22, 26, 48-50)

These electrophysiological studies describe the following :

- Increased mean N1 amplitude (49)
- Trend towards reduced N1 habituation (9)
- Increased (hand) mean N2-P2 peak-to-peak amplitude (9, 10, 22, 26, 49)
- Decreased (hand and foot) mean N2-P2 peak-to-peak amplitude (7, 17)
- Reduced N2-P2 habituation (all sites) (9, 17, 50)
- P3b waves (22)
- Reduced mean pain threshold (hand) (10, 49)
- Increased subjective laser pain (all sites) (7, 9, 50)

Unfortunately, data cannot be directly compared between those 8 studies, because of :

- Different laser stimulation methods (CO_2 laser, YAG laser, Thulium laser)
- Different number of stimuli per stimulation site
- Different inter-stimulus intervals used (35)

Also, the majority of these studies had some or both of these disadvantages

- Only a small group of patients was examined (7)
- There was no control group (healthy subjects)

2.2.8.4 Identification - and influencing factors of the laser-evoked potential components

(9, 15, 18, 36, 39, 47, 51)

First, the *N1 component* was defined as the negative deflection preceding the main evoked complex (N2-P2) (Fig.9). It is the earliest and therefore first LEP response to laser stimuli, emerging after stimulus onset between 100 and 300 ms in healthy individuals (51). It appears as a small shoulder in the N2 ascending slope, but compared to the latter, it is far smaller in amplitude and lateralized more bilaterally on scalp topography. Its distribution is maximal over the temporal region contralateral to the stimulated side: i.e. electrode T3 after right - and T4 after left stimulation. Most importantly, it is the sole pre-perceptual response in LEP recording: insensitive to attentional modulation and resistant to cognitively induced manipulation of pain sensation. There is growing experimental evidence indicating that the N1 wave is directly related to the ascending nociceptive input. Therefore, it should be systematically included in the assessment of sub- and early cortical pain processing. Though, there are some concerns to be taken into account: the overlap of N1 and N2 waveforms in time and - in space (opposite polarities), as well as temporalis muscle artefacts contaminating the temporal electrodes, may cause N1 waves hard to reproduce. Also, the N1 is known to have a high inter-subject variability, due to the fact that it is generated by neural activities of smaller magnitude than those underlying other waveforms and thus has a smaller signal-to-noise ratio (SNR). This low reproducibility and high inter-subject variability can be countered by applying strong stimuli, multiple trials, reference of T3/T4 to Fz (temporal-frontal montage) and trace superposition.

Next, the biphasic N2-P2 complex consists of the following two components. The *N2 component* is the negative deflection preceding the *P2 component*, which is the first positive deflection of the main laser evoked potential (Fig. 9). They both reach their maximum peak-to-peak amplitude (several tens of microvolts) at the vertex (electrode Cz), and occur between 200 and 350 ms after stimulus onset in healthy individuals (51). The major importance of the N2-P2 complex lies in its exclusive relation to the Aδ-nociceptor activation, as described by Bromm et al (1987), and reflects the neural activities for the sensory modality of the elicited stimulus (52). This main LEP complex expresses the attentive orientation towards - and the salience of the noxious stimuli, i.e. the quality by which it stands out relatively to other perceived stimuli. This detection phenomenon enables the individual to focus their limited cognitive resources on only the relevant subset of all presented sensory data, following a hypothesis described by Berridge and Robinson in 1998 (53). Initial research showed a reduced P2 amplitude with attention. Followed by Legrain et al (2002) who reported that the P2 component is never affected by (spatial) attention, but can be modified by the probability of the stimulus (36). Treede et al (2003) pinpointed that the N2-P2 peak-to-peak amplitude will augment accordant to increased selective attention (focused subject), only if the ISI is less than 3 seconds (15). Therefore, it is recommended to use a sufficient long ISI, to make the recruitment of spatial attentional mechanisms impossible as early as the N2-P2 components. However, a natural decrease in the N2-P2 amplitude in function of age is a consistent influence factor; this was objectified in healthy individuals.

Finally, the fourth component of the laser evoked potential, only visible in attended conditions, is the *P3 positive deflections*, generally subdivided in a P3a and P3b peak (Fig. 9). It can be studied at electrode Pz (parietal), between 300 and 500 ms after stimulus onset (51). This component appears rather as a small shoulder in the P2 slope when returning to baseline. P2 modulations are similar to those observed for in P3a, and the latter is therefore often difficult to distinct on its own. Alas, Legrain et al (2002) could not establish whether the laser-evoked P2 peak shares common processes with the P3a peak. However, the P2 is definitely not a P3b-like component (36).
Elicited by rare - (or novel) stimuli, the P3a reflects an orientation response (an involuntary switch of attention to the unexpected event), the P3b the conscious detection of this target. The latter reflects the resetting of cortical structures involved in target perception control, updating of the working memory and closure of the contextual processing. It is under debate whether the subject deliberately or unintentionally responds to these stimuli. If deliberately, the P3b component, indicator of the sensory stimulation reaching the patient's awareness, would correspond to the phenomenon of malingering (Lorenz et al 1998), i.e. the simulation of sensory loss with normal sensory-perceptual components (48). In conclusion: the P3b -, but not the P3a component, is probably influenced by intrinsic, non-nociceptive, cognitive factors such as attention, arousal or anxiety.

2.2.8.5 Terminology and definitions

(9, 26, 47, 51)

The different LEP waves are generally characterized based on their latency relative to stimulus onset, their polarity, their amplitude and their scalp topography, which can then be compared to that of normative data.

The scalp potential *latency* refers to the length of time (in milliseconds) passed after stimulus onset to the maximal (i.e. highest) peak. Due to a longer peripheral stimulus travel distance, the N2-P2 latency is greater after foot - compared to hand stimulation, with a peripheral nerve conduction velocity of the Aδ pathway in the order of 10 m/s. Controversially, most authors agree that age has no significant effect on the LEP latency after nor hand, nor foot stimulation. Opposite, there's an obvious correlation between the limb-LEP latency and the body height (and thus the conduction distance). This can be described by a steep linear function with wide latency changes, due to the low conduction velocity of the pathways. Truini et al (2005) recommend therefore a necessarily height adjustment in clinical practice for both foot - and hand-LEP (31). Once matched for height, the latency of the LEP of males and females are almost identical. Of course, due to this longer peripheral stimulus travel distance, the latency of the LEP peaks determined will always be later for foot - than for hand stimulation. Lastly, a longer stimulus duration equals a longer LEP latency.

Peak *duration* is defined as the side-to-side difference in time of the begin of one deflection and the end of the consecutive deflection.

Peak *amplitude* is described as the voltage difference between two consecutive LEP peaks, or between the LEP peak and the baseline. The unit of measurement is microVolts (µV). The baseline is defined as the averaged amplitude of the pre-stimulus interval (500 ms long). The amplitude is directly depending on the number of healthy neurons in the LEP generating brain areas.
Research found consistently that in healthy subjects the LEP amplitude negatively correlates with age, i.e. an age-related decrease in LEP amplitude, sometimes even to an extent that the suspected

evoked potential is absent. Supporting this statement, a relatively high percentage of bilaterally absent foot-LEP was found in elderly subjects. Supposedly, with advancing age comes a subclinical, mild neuronal loss or dysfunction in the PNS or CNS, resulting in a reduced afferent input. Furthermore, Truini et al (2005) found a distance-related decrease of the LEP amplitudes (31). A long conduction distance goes accompanied by a high signal dispersion along the Aδ-afferents, followed by a desynchronized volley presented at central synapses. This higher signal dispersion together with a lower free nerve endings density distally, could explain the smaller amplitude responses after foot stimulation compared to equal stimulation at the hand side.

Peak *habituation index* (HI) is the computed quotient between the LEP amplitudes obtained in the third and first block of evoked responses, but can also be calculated otherwise (9). This ratio shows us the relative decrease due to the habituation phenomenon.

Habituation is the physiological response to repetitive similar stimuli, which install a progressive peak amplitude decrement, resulting in the advancing reduction of the neuronal activation of the sensory cortex; the latter exists to avoid brain overstimulation. The response recovers after stimulus detainment. A higher frequency of stimulus application or weaker stimuli will provoke quicker habituation. This phenomenon is always present in normal, healthy circumstances, except in cases of receptor fatigue. Extraneous stimuli can result in a abnormal recovery of the attenuated responses (dishabituation). Reduced habituation of cortical responses to laser stimuli suggest alterations in the pattern of cortical excitability.

2.3 Aims of this study

The aim of the present study was to investigate the usefulness of LEP as a diagnostic tool for dysfunction of the nociceptive system in the context of FMS. Abnormal LEP findings in a large cohort of FMS patients would suggest an underlying pathophysiological mechanism of this invalidating chronic pain syndrome in relationship with a lesion or dysfunction of the central and/or peripheral nervous system. LEP are the best available diagnostic tool (non-invasive, unbiased, objective, quantitative, sensitive, specific) for exploring the thermo-nociceptive nervous system (32). To avoid the bias of visual analysis of LEP, an automated analysis of LEP was used according to the detailed description by Hu et al (2010) (see section 3.4.2.2) (39).

The primary endpoint of the present study is to describe LEP findings in a large cohort of FMS patients. Till now, it is described that FMS patients can be classified in (1) patients presenting with central sensitization phenomenon and (2) patients with lesions of the peripheral nervous system similar to small fibre neuropathy. The existence of these patient subgroups will be verified on a large sample.
The secondary endpoint is to derive suggestions for the pathophysiological mechanisms underlying FMS, based on the LEP abnormalities observed in our large FMS cohort.

In conclusion: this research aims at examining the relevance of LEP as a diagnostic tool for FMS and deriving possible pathophysiological mechanisms explaining neuropathic symptoms in this chronic pain syndrome.

2.4 Hypotheses

- Some of the pathophysiological mechanisms of FMS include: central sensitization and/or peripheral nociceptor spontaneous discharges. It is hypothesized that LEP findings in a large cohort of FMS patients would corroborate one or both of these suggested pathophysiological mechanisms involving the thermo-nociceptive nervous system.
- Previous microneurographic and evoked potential data indicate that some patients with FMS may actually have small fibre neuropathy. It is hypothesized that part of the FMS patients in this large cohort will show LEP findings compatible with small fibre nerve dysfunction.
- The influence of demographic factors, such as sex and age, will be examined on the function of the thermonociceptive nervous system.
- Data of different examination sites will be compared in order to determine which body location may be regarded as the most appropriate to diagnose thermonociceptive dysfunction in FMS.
- Two previous studies have used the LEP habituation index as a diagnostic tool for central sensitization in FMS. The presence of LEP habituation abnormal findings in this large patient cohort will be investigated and compared with the presence of abnormal LEP amplitudes (increase).
- The latencies and amplitudes of LEP peaks in FMS will be compared to a group of healthy controls in order to determine which peaks are mostly pathological in FMS.

3 Methodology

(Time course: see Appendix 1)

3.1 Subjects

3.1.1 Patient file selection

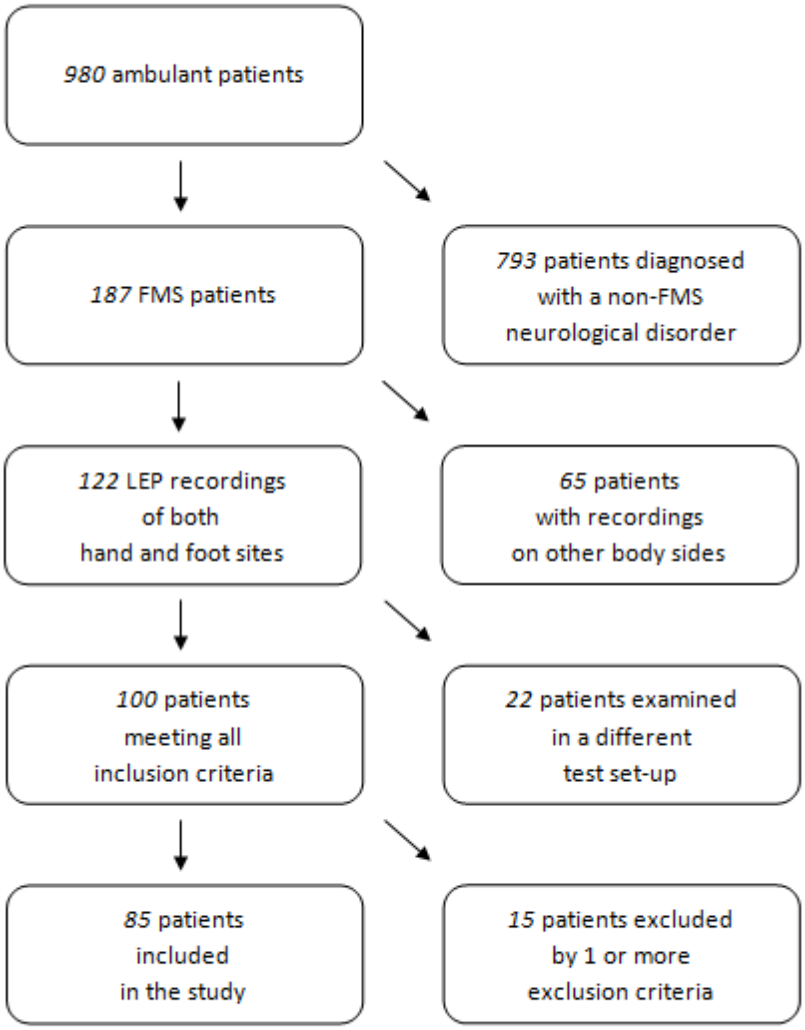


Fig. 6 *Enrolment of subjects*

One thousand four hundred and forty (1440) paper and electronic files concerning nine hundred and eighty (980) different out-clinic patients, examined with LEP by L.P. at the Laboratory of Algology in the department 'Systemic and cognitive neuroscience' (COSY) of the Institute of NeuroScience (IoNS), of the 'UCL', Brussels, Belgium, between January 2nd 2003 and December 30th 2012, were screened for inclusion in this retrospective observational study.

All relevant demographic, clinical, psychophysical and electrophysiological data of all 1440 patient files were collected in an excel data file.

Following careful screening of these 1440 patient files,, it was determined that patients had a diagnosis of FMS at the time of electrophysiological testing, based on the following criterion: clinical diagnosis of FMS by the referring physician according to the 1990 ACR diagnostic criteria (widespread pain for more than 3 months and tender points > 11/18) (Fig.6).

An information sheet and informed consent was prepared to be sent to patients at their request (Appendix 4).

Referring physicians were contacted to collect demographic and clinical data on the patients selected from the Algology Laboratory database. Referring physicians mainly were rheumatologists and specialists in physical medicine and rehabilitation working at Cliniques universitaires St-Luc (Brussels) and CHU Montgodinne (Liège). They provided minimal clinical information on the past medical history and current symptoms from their medical records. This procedure was meant to ensure an accurate patient diagnosis and to check the predefined inclusion - and exclusion criteria. Tables 10 and 11 (see section 4.1) describe these collected data.

3.1.2 Inclusion criteria

1. FMS diagnosis according to the 1990 ACR criteria, confirmed by the referring physician based on the past (at the time of referral for electrophysiology) and present medical condition

2. Over 18 years of age

3. Electrophysiological data of both a hand - and a foot recording (ipsi- or contra-lateral)

4. Standard CO_2-laser settings (as described in section 3.2)

3.1.3 Exclusion criteria

1. Other known (co-existing) neurological – and/or other disorders, which could explain the patient's symptoms, e.g. neuropathies

2. Incomplete LEP recordings (only hand or only foot) or LEP recordings of poor quality, especially those with a low SNR ratio

3. Brain disease in the patient's medical history, prior to the LEP examination

4. The use of CNS-acting drugs and analgesics at least 24h prior to the LEP examination, which could possibly interfere with the quality and interpretation of EEG data; e.g. Benzodiazepines (54). Patients received clear instructions in advance to avoid the intake of those drugs. Unfortunately, it cannot be excluded that some patients did use psychotropic drugs at the time of recording.

3.1.4 Control groups

The database of the Algology laboratory at UCL contains control data of healthy subjects that were examined by using the exact same standardized LEP recording parameters (number of stimuli on each site, laser stimulus intensity and duration,...) and acted as a control group for previously published studies (16, 35, 55).

The LEP recordings on the hand dorsum of the FMS cohort were compared to those of a group of 21 age- and sex-matched healthy individuals, participating in a clinical study by Hatem et al (2010) (55). Demographic - and LEP data of this control group can be found in section 4.1 (Table 2 and 3) (55).

The LEP recordings on the foot dorsum of the FMS cohort were compared to the distal leg recordings of a group of 18 age- and sex-matched healthy individuals, participating in a study by Ragé et al (2011). Demographic and LEP data results can be found in section 4.1 (Table 2 and 3) (35).

3.1.5 Ethics commission

This study was conducted in accordance with the Declaration of Helsinki. All research procedures and protocols (Appendix 2) were submitted and approved by the local Ethics Committee of Cliniques universitaires St-Luc, as well as the Ethics Committee of CHU Brugmann; Brussels, Belgium. Their approval can be found in Appendix 3.

3.2 EEG experimental setup and laser-evoked potential recording procedure

This study is a retrospective study on electrophysiological data acquired over a 10-year period. Throughout these years, all LEP assessments were done by the same investigator (L.P.) in standardized technical circumstances and thus recording methods remained robustly unchanged. This is the reason why it is possible to describe with maximum reliability how the LEP recording procedure was performed and to concatenate electrophysiological data recorded over such a long period of time.

3.2.1 Subject positioning

All subjects were informed about the technical aspects prior to starting the LEP recording. They were warned that in few cases a transient skin rash of the stimulated region may occur. No other side effects are observed.

The subject was placed awake and relaxed with their forearms resting on arm rests of a comfortable chair in a quiet, air-conditioned room with an ambient temperature of 22-24°C. The total duration of LEP recording was 1h.

All equipment associated with the production of the laser stimulus was kept outside of the visual field of the subject.

Subjects were instructed to maintain their eyes open and gaze steady (fixation cross) to avoid signal contamination with ocular movements, eye blinks and eyes-shut EEG alpha activity.

Also, subjects were instructed to pay attention to stimuli delivered, and to press a button (micro-switch) as soon as they became aware of any type of sensation at the stimulation site. The time between the opening of the laser shutter and the pressing of the micro-switch was automatically recorded as the reaction time (RT) (56). The detection rates (i.e. the number of laser stimuli correctly identified by the subject) were deduced from these RT. When the RT exceeded 2500ms, the stimulus was considered as undetected (35). In this manner, attentional and vigilance conditions were precisely standardized and biases restricted to the utmost minimum.

Cutaneous heat stimuli were delivered by a CO_2-laser beam at the right or left hand dorsum of the subject, followed by a series of laser stimuli at the ipsilateral or contralateral foot dorsum.

3.2.2 Placement of electroencephalography electrodes

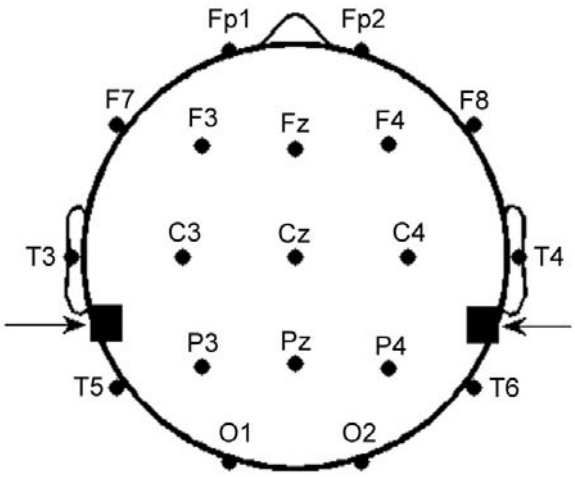


Fig. 7 *Cranial view of the scalp designating the positions of the 21 electrodes in the 10–20 international System for electroencephalography (EEG).*
(odd numbers indicate leads on the left -, even – on the right side, Z indicates the zero -/midline, F = frontal, C = central, P = parietal, O = occipital, T = temporal)
[Adapted from Kim et al (2013) (57)]

Nineteen Ag-AgCl cutaneous scalp surface electrodes were positioned according to the International 10–20 System of EEG electrode placement (i.e. the most widely used method in clinical studies), referenced to the earlobes (reference electrodes: A1, A2), with the active electrodes in positions: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2. Special care was taken in the placing the scalp electrodes, to avoid recording artefacts. The impedance was kept below 5 kΩ by applying a sufficient amount of electrode gel. In addition, an electro-oculogram (EOG) of the right eye was recorded with 2 disposable Ag-AgCl surface electrodes (up and below eye), to monitor ocular movement – and eye blink artefacts (channel Poly1). The ground electrode was placed at the unstimulated fore-arm. (15, 16, 35, 51, 58)

3.2.3 CO_2-laser properties

(15, 16, 20, 26, 31, 35, 36, 55, 56, 58)

General considerations on the use of LEP are described in section 2.2.8. The following section contains a more detailed description of the stimulation properties and specific interest of the CO_2-lasers.

Particularly CO_2-lasers have interesting properties for clinical use. The CO_2-laser has an advantageous wavelength of radiation (10.6 µm), which closely matches the thermophysical properties of the skin, and limits absorption of thermal energy within the first 100 µm depth of the superficial skin layers (Table 6). It can activate the nociceptors in a few milliseconds, due to its high thermal power, thereby focussing the stimulus accurately in time. (Table 6) The almost complete (>99%) absorption of the thermal energy and a negligible skin reflectance in the epidermal layers guarantees the reproducibility of the stimulus. Furthermore, the absorption is independent of pigmentation of the skin. Also, the laser can be applied to the non-glabrous skin in any dermatome. Finally, the CO_2-laser is the most reliable method in the clinical and experimental assessment of the nociceptive system (32). It even has the potentiality for detecting changes over time. It is non-invasive, quantitative, sensitive and specific. It is the method of choice to examine lesions of the nociceptive somatosensory system which may occur in FMS.

The CO_2-laser device used in for the electrophysiological assessments described in this research study, was designed and built in the department of physics of the UCL (Brussels, Belgium).

Table 6 *Laser properties in this research setup for LEP recordings in FMS - and healthy control subjects (35, 55)*

	FMS patients	Hand controls	Foot controls
Reference	-	(55)	(35)
Wavelength (µm)	10.6	10.6	10.6
Surface area (mm²)	80	80	20
Impedance (kΩ)	<5	<5	<5
Beam diameter at target (mm)	10	10	
Energy density (mJ/mm²)	9.3 ± 1.3	9.6 ± 0.9	10
Intensity (W)	7.5 + 2	low	low

The power output was determined such that energy density (mJ/mm²) remained supraliminal for Aδ-nociceptor activation. The Aδ-fibre activation threshold in healthy controls is usually around 5.7 ± 2.6 mJ/mm² (hand) and 7.3 ± 4.4 mJ/mm² (foot), described by Truini et al (2005) (31). The same energy density was used at both stimulation sites. The laser stimulus intensity used for evoked potential recording was slightly above the Aδ-fibre activation threshold of each patient. The determination of the Aδ-fibre activation threshold and the stimulation intensity is important, since it is an extrinsic factor that may influence significantly the morphology and topography of the final LEP waveform (as well as other stimulus characteristics).

Thermal drift of the laser equipment is a natural phenomenon frequently described in studies. It consists in a natural increase in energy level of the laser device of about 7% between the beginning and end of a laser stimulation session. To avoid a possible stimulation bias by thermal drift, the laser device was ignited approximately 30min before the start of each LEP recording session.

A CO_2-laser infrared heat pulse is not visible for the human eye. Therefore, a He-Ne laser beam, with a wavelength in the visible red spectrum, is superimposed on the CO_2-beam, making it possible to precisely visualize the location of the laser stimulus in the body. At each stimulation site, 30 laser stimuli (in 3 consecutive series of 10 stimuli) were applied in order to obtain sufficient electrophysiological data for averaging.

3.2.4 Brain-potential registration specifications

(15, 16, 26, 31, 35, 36, 51, 58)

EEG recordings were acquired using the Signal Software (Version 2.16).

The stimulation sites were: the right or left dorsum of the hand, and the contralateral or ipsilateral dorsum of the foot, in all subjects. The order of site stimulation was first hand, followed by foot stimulation. Only unilaterally stimulation was performed, as FMS typically is a symmetric disease.

LEP, EOG, RT and laser trigger signals were amplified and digitized, using a PL-EEG device (Walter Graphtek, Germany) (35, 51).

Table 7 *Processing features in this research setup for LEP recordings in FMS - and healthy control subjects*

Gain setting	x 1 000
Bandwidth	1 – 1 000 Hz
Band pass filter	0.06 – 75 Hz
Sampling rate	167 Hz
Measurement resolution	10 bit
Notch filter	50 Hz

Previous to the recording session, a first series of 5 laser stimuli at gradually increasing and decreasing power intensities (steps of 1.5 W) was presented to the area of interest in order to familiarize the subject with the laser stimuli and to determine the laser detection (or perceptive) threshold (DTh) and the laser pain threshold (PTh). The PTh is defined as the lowest intensity of laser stimulus that evokes a pin-prick sensation (Aδ-nociceptor related activation) followed by a burning sensation (35). The DTh was determined as the lowest intensity at which the subject perceived at least 50% of the laser stimuli (C-fibre related activation). (31) The mean PTh of healthy subjects was determined at 5.7 ± 2.6 mJ/mm² (hand) and 7.3 ± 4.4 mJ/mm² (foot), as described by Truini et al (2005) (31). For LEP recording, a stimulus intensity slightly above the DTh (about 20 to 30%) was used.

Table 8 *Measurement properties in this research set-up for LEP recordings in FMS - and healthy control subjects*

	FMS patients	Hand controls	Foot controls
Examinator	LP	SH	MR
Reference	-	(55)	(35)
Number of patients	85 Hand / 85 Foot	21	18
Recording location	Right or Left, ipsi- or contralateral dorsum of hand and foot	Dorsum of the hand	non-dominant calf (10cm above the lateral malleolus)
Stimulus duration (ms)	50	50	50
ISI = interstimulus interval (s)	Pseudo-randomly varying between 5-10	Pseudo-randomly varying between 5-10	6-12
Number of trials / stimulation side	Min 30	Min 30	Min 30

Subsequently, subjects were exposed to 3 consecutive series of 10 laser stimuli per stimulation site (60 laser stimuli in total). Each laser stimulus was followed by a variable inter-stimulus interval (ISI) of 5-10 seconds and a few minutes rest was observed after a series of 10 stimuli. It was important to take an ISI of 'long' duration (>3s), to avoid spatial and temporal habituation to the laser stimulus. (Table 8)

The target spot was repositioned slightly after each stimulation, pseudo-randomly, to avoid skin damage due to overheating, to minimize central habituation, and to fence of nociceptor sensitization or – fatigue.

Next, EEG signals were amplified and stored on a hard disk, for off-line analysis in BrainVision Analyzer 1.05 (Brain Products GmbH, Germany) (35). Data were transferred for further management to a MATLAB application: Lets Wave EEG toolbox version 5 (http://www.nocions.webnode.com/letswave) (44). The accuracy of the data entry was verified.

3.3 Recordings pre-processing

3.3.1 Visual analysis in BrainVision

The offline signal-processing steps were performed 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 at 0 ms (512 data points). Technical - and blink artefacts (EOG-contaminated sweeps) were rejected after visual inspection. A band pass filter of 0.3-20 Hz (80 dB/decade) was applied, followed by time-averaging of the epochs, in order to enhance the SNR. Next, in each channel the sweeps were corrected for baseline offset, based on the 500 to 0 ms during pre-stimulus interval. Finally, for clinical purposes, L.P. visually identified and characterized the LEP peaks in each averaged waveform, as described in section 3.4.1. These results can be consulted in Table 10 (hand) and 11 (foot) in section 4.1. (16, 35, 39, 44, 51)

3.3.2 Automated analysis in LetsWave-Matlab

For the present study, instead of using the LEP data obtained by visual analysis over a ten-year period by L.P, all original raw data were extracted from BrainVision and re-analysed with an automated signal-processing procedure. This method allowed to (1) homogenize the pre- and post-processing of all EEG data and (2) to avoid the investigator bias inherent to a visual analysis of LEP peaks.

The raw data were re-analyzed in the present study, using the open source Lets Wave EEG toolbox, version 5 (LW5), running under the MATLAB environment (http://www.nocions.webnode.com/letswave) (44). Only the investigator who performed the electrophysiological recording (L.P.) was aware of the patients' diagnosis. All original raw data were coded in order to ensure blinding (for the clinical diagnosis) of the investigator running the automated analysis. Thus, the present investigator (D.V.A.) , was at the time of the pre- and processing of raw data, unaware of the name and clinical diagnosis of subjects.

EEG epochs were extracted using a time window for analysis of 4 000 ms, ranging from 1 000 ms pre-stimulus to3 000 ms post-stimulus, relative to stimulus onset at 0 ms (668 data points).

Different offline signal-processing steps were implemented, being: channel removal, corrupted epoch removal, band-pass filtering, baseline correction, artefact rejection by independent component analysis (ICA), and re-referencing of the temporal – to the frontal electrodes; followed by objective estimation of the LEP peak characteristics through automated single-trial analysis (ASTA), as described by Hatem et al (2012) (51). The latter will be specified in section 3.4.2.2.

3.3.2.1 Channel removal

The Poly 2 (stimulus onset) and Poly 3 (micro-switch) electrode channels were removed from the signal data in all epochs, as they contained non-physiological signals, solely used for synchronization of the stimulus with the recording device.

3.3.2.2 Epoch removal

Due to the technical boundary conditions, rare epochs may get corrupted. This can be recognized by a time window larger than 100 ms in which no signal information is present (i.e. a perfect constant signal line over time). These epochs were filtered in any recording channel from the data set. In 12 patients at least 1 epoch had to be removed due to a technical artefact in the hand stimulated trials, and in 5 patients in the foot stimulated trials.

3.3.2.3 Band-pass filtering

The EEG data was subjected to a 4th order Butterworth band-pass filter between 0.3 and 30 Hz, as proposed by Hu et al (2010) (39).

3.3.2.4 Baseline correction

The pre-stimulus time interval reaching from -500 ms to 0 ms, was used as a reference for estimating the baseline offset. This estimated offset was subtracted from all data (39).

3.3.2.5 Artefact rejection

(39, 51, 59-61)

ICA, a validated method using spatial filtering, was applied to subtract electro-oculographic and electro-cardiographic artefacts.

This validated method uses the ICA algorithm of Bell and Sejnowski (1995) with the natural gradient feature of Amari, Cichocki and Yang (1996) and the extended ICA algorithm of Lee, Girolami and Sejnowski (1999) with optional PCA dimension reduction.

First, an ICA transformation matrix was built based on all epochs and channel data, creating independent components (ICs). A linear combination of these ICs can approximate every channel in every epoch.

ICs related to ocular movements and eye blinks had a large contribution in the EOG channel (Poly1) and in the frontal scalp channels (Fz, Fp1, Fp2). These interfering movements were clearly visible in the ICs. In both channels, if contaminated, the ICs were deleted and ICA inverse filtering was applied.

An example can be seen in fig. 8 where IC 1 contains a 'blink' artefact in channel Poly1 of epoch 1. The LW5 tool provides the means to display the original channel data (black) and its filtered version after removal of the contaminating IC (red).

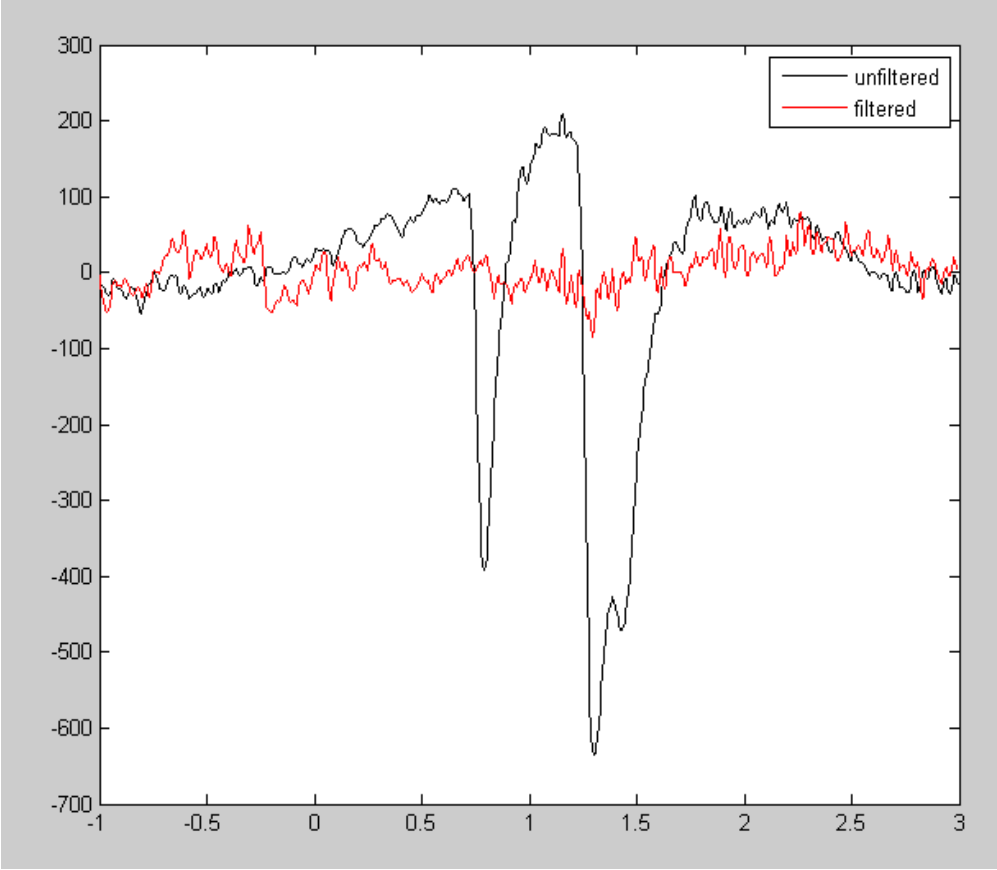


Fig. 8 *Display of the original signal (black), contaminated with ocular movements, and filtered signals after IC1 removal (red) of only epoch 1 in channel Poly1 - The X-axis is the time-course (in ms), the Y-axis the signal level (in µV)*

3.3.2.6 Re-referencing to the frontal central electrode

The N1 deflection is mainly visible in the temporal electrodes (T3, T4) and suppressed in the frontal electrode (Fz). To isolate the N1 wave-form from the N2 wave-form, the N1 peak was detected in the contralateral temporal electrode referred to the frontal central electrode (15, 17, 39).

3.4 Data analysis

3.4.1 Laser-evoked potential components

(9, 15, 16, 20, 31, 35, 36, 40, 42, 43, 47-50, 55, 58, 62-64)

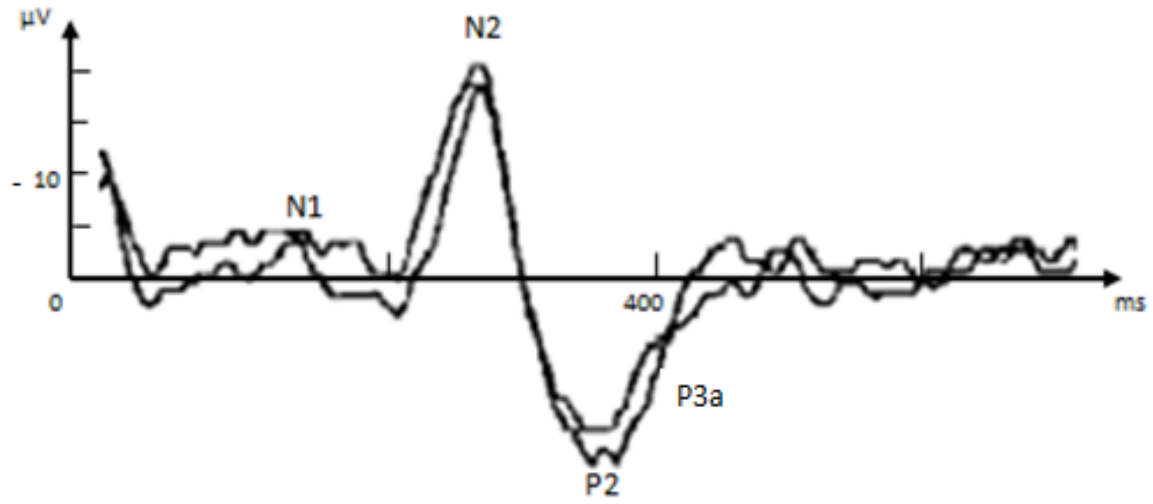


Fig. 9 *Typical waveform of laser-evoked potential recording in a healthy control as seen on channel Cz. Single trial epochs were recorded, referenced to linked earlobes (A1-A2), baseline corrected, artefact rejected and averaged after hand stimulation. The LEP components are shown on the waveform (N1, N2, P2). The X- axis represents time from stimulus onset and the Y-axis the amplitude of the waveform (defined as a voltage difference). [Adapted from Truini et al (2005) (31)]*

Brain activations, subsequent to laser stimulation of the skin, can be recognized as electrical activity on the scalp, shown as deflections as described in fig. 9. Usually in LEP recordings, three different well-known components, are observed: N1, N2 and P2. A fourth component, labelled P3, may be observed in specific recording conditions. Further details can be found in section 2.2.8.4 and 2.2.8.5.

In the present study , the three main components were characterized for each subject and each waveform. The most prominent cortical response usually measured in a clinical setting, is a widespread negative-positive complex, composed by a negative deflection (N2), followed by a positive deflection (P2). Neurophysiological convention describes negative potentials as upward - and positive potentials as downward deflections. We labelled the 3 LEP responses according to a procedure of Valeriani et al (18) Furthermore, the habituation index was calculated as described in section 4.6.1 (see also section 2.2.8.5).

3.4.2 Automated single trial analysis

3.4.2.1 Rationale for using an automated single trial analysis

Until recently, the visual inspection has been regarded as the only widely available and generally agreed-on method to identify the different waves of LEP. However, Hatem et al (2010) exposed the following limitations of a visual identification of LEP (55).

- the interpretation of the different LEP components is necessarily observer-dependent, and therefore, should be performed blinded.
- the value of the characterization of LEP becomes doubtful, when at least one defining criteria is not fulfilled (absence of a peak, latency outside of the usual time window, ...).
- in a group-analysis, LEP waveforms with no (visually) detectable peak, can neither be discarded, which would bias the group-level results (overestimated mean amplitude), neither be modified by arbitrarily allocation of a zero magnitude to the missing values, which would give the opposite (an underestimation of the mean group amplitude).

Nevertheless, in a clinical context, visual inspection of LEP should not be abandoned, because it is likely that the trained eye of the expert integrates important features of the waveforms that are not taken into consideration by the automated analysis.

In 2010, Hu et al described a novel automated method to perform LEP analysis, which could identify reliably the LEP characteristics in a healthy subject population, with a similar sensitivity and specificity as the visual analysis (39).

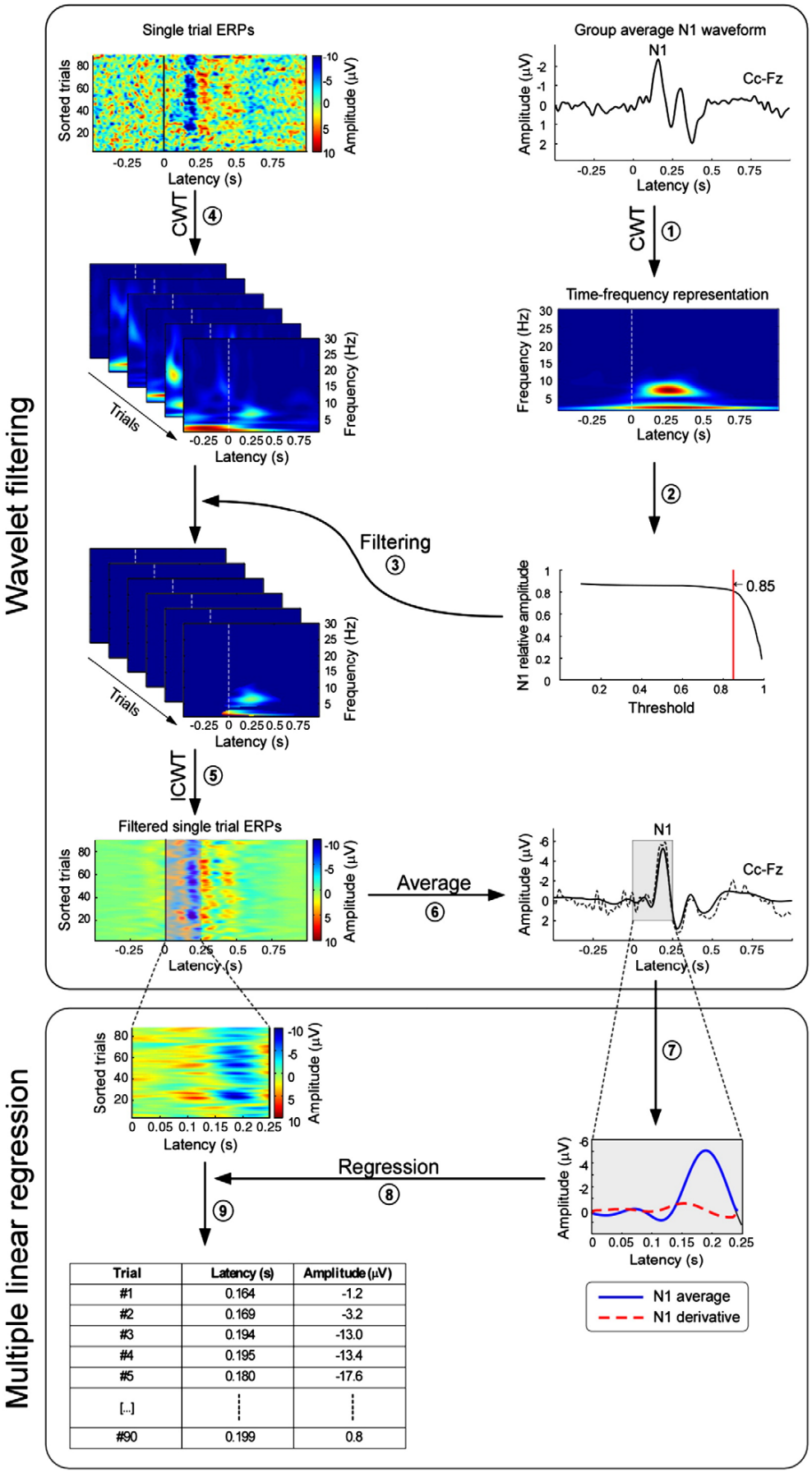
Hatem et al (2012) showed a high absolute agreement between the visual and automated analyses in a patient population, especially for amplitude measurements (51). Even more, in a fraction of trials, the automated analysis could identify, LEP that remained undetected visually in the averaged waveforms.

ASTA was found to be a highly useful tool to compare patient groups in clinical research, and presents several advantages compared to visual identification of LEP peaks. First, the estimated peak values are entirely independent of the subjective interpretation of the observer; there is no inter-researcher variability, nor difference in the determined values by the same researcher in different days. The estimates obtained with an automated analysis are reproducible and comparable across experiments and laboratories. Second, the magnitude of event-related EEG responses is several factors smaller than the magnitude of the background ongoing EEG. Therefore, the identification and characterization of LEP rely on signal processing methods for enhancing their SNR. Third, some patients exhibit LEP waveforms that are intrinsically difficult to recognize (due to abnormal amplitudes, latencies or morphology). As shown by Hatem et al (2012), ASTA does not consider any a priori knowledge of the waveform shape, hence, it is capable to analyze any input, even those which could be labelled as corrupted by visual analysis. Fourth, when the stimulus does not elicit any evoked cerebral response, the across-trial average of the single-trial estimates of amplitude will tend towards zero. Even in the presence of a barely-detectable response, the across-trial average of the single-trial estimates of amplitude will be different from zero, thus providing a reliable estimate of the amount of stimulus-evoked response in the recorded trials. single-trial automated analysis assigns a value to the LEP peaks of each waveform. Hence, missing values are avoided and the statistical analysis is more powerful. Fifth, automated analysis is less time-consuming than visual inspection (51).

The automated single-trial analysis can be used to characterize normal and abnormal LEP with a similar sensitivity and specificity as the conventional method based on visual inspection. Based on previous arguments, we can conclude that this automated approach is a reliably (unbiased and objective), accurate and efficient alternative for visual analysis of LEP and for exploring possible dysfunctions of the nociceptive system.

3.4.2.2 Automated single trial analysis: technical aspects

In 2010, Hu et al described an approach for automated LEP peak estimation, through across-trial averaging in the time domain (39). This currently common technique is founded on ICA, followed by a combination of wavelet denoising and multiple linear regression analysis, to estimate the LEP characteristics. The method is summarized in the flowchart in figure 10: it can be subdivided in a SNR enhancement of the wave (top panel), and automatic measurement of its peak latency and amplitude in single trials (bottom panel). Furthermore, this procedure can be executed in user-friendly software running under the MATLAB environment, and can be freely downloaded from http://iannettilab.webnode.com . (16, 36, 39, 51)



Trial	Latency (s)	Amplitude (μV)
#1	0.164	-1.2
#2	0.169	-3.2
#3	0.194	-13.0
#4	0.195	-13.4
#5	0.180	-17.6
[...]	⋮	⋮
#90	0.199	0.8

Fig. 10 *schematic overview of ASTA.* *[Adapted from an article by Hu et al (2010) (39)]*

3.4.2.2.1 Wavelet filtering

(39, 44, 47, 51, 58)

An obtained waveform expresses its average scalp potential as a function of time relative to the onset of the sensory event. To ground this procedure, the following assumption must be made: LEP are stationary (i.e. their latency and morphology are invariant across trials), time-locked to stimulus onset, consequently they will remain unchanged after averaging, while the ongoing electrical brain activity will behave as noise unrelated to this event, occurring randomly, and therefore it will mostly be wiped out by an averaging procedure of repeated responses, thus enhancing the SNR of the LEP.

Wavelet filtering is performed to reduce background noise as well as a part of the N1 or N2-P2 response, in order to increase the SNR of respectively the N2-P2 or N1 response. Due to the nature of EEG signals, in which high frequency peaks only occur in limited time intervals, wavelet filtering is well suited for noise rejection. The applied strategy is displayed in fig. 10 (top panel).

First, the averaging of the different epochs is calculated for each channel. Based on this average, a wavelet filter (CWT = continuous wavelet transform) is used to decompose the averaged LEP into a time-frequency domain representation. The same transformation is also administered in parallel to each single-trail. The time-frequency representation offers an optimal compromise for time and frequency resolution by adapting the window width as a function of estimated frequency. Furthermore, a threshold for the average time-frequency matrixes is set with the objective to retain wavelet coefficients with high energy and to eliminate wavelet coefficients with low energy. Regions above a relative contribution threshold of 0.85 in the time-frequency representation of the averaged wave are preserved in the time-frequency representation of every single trial. Next, an inverse continuous wavelet transformation (inv-CWT) reconstructs this set of modified time-frequency representations in the time-domain. Finally, the modified single trials were averaged to serve as the input to the multiple linear regression (MLR).

Specifications about the mathematical background or a more detailed explanation regarding these procedures can be found in the article by Hu et al (2010) (39).

The cost of the across-trial averaging procedure is that all the information concerning across-trial variability of LEP latency and amplitude is lost. However, this variability could reflect important factors such as differences in stimulus parameters (duration, intensity, and location), and most importantly, fluctuations in vigilance, expectation, attentional focus, or task strategy. However, the simultaneous occurrence of these evoked potentials with spontaneous potentials, other biological potentials and ambient noise, make signal averaging indispensable.

3.4.2.2.2 Multiple linear regression

The MLR was used to obtain an automatic, fast and unbiased estimation of the peak amplitude and latency of the LEP waves at the level of single trials.

Table 9 displays the different LEP peaks defined with this method, as well as the corresponding channels and the time intervals in which they were determined.

MLR analysis is applied as a means of estimating the peak latency and - amplitude in each single trial, after SNR enhancement. Regression is performed with respect to the average of the modified single trials and its time-derivative in a time-window as summarized in Table 9.

Table 9 *Different LEP peaks defined with ASTA*
[based on proposals made by Hatem et al (2012)]

	Observed channel	**Post-stimulus interval**
N1	T3 (controlateral to the right side) T4 (controlateral to the left side)	0.1 - 0.33
N2	Cz	0.1 - 0.52
P2	Cz	0.16 - 0.71

The linear regression model can be written as:

$$Y = \sum_{i=1}^{2} (\beta i \, Xi + \varepsilon)$$

where Y is a matrix containing all epoch data points, Xi are the regressors (i = 1 is the average and i = 2 is its time-derivative) and βi are the weight factors.

A repressor and its central or temporal derivative (based on post-stimulus interval as described in Table 9) are obtained from the across-trial average waveform measured after wavelet filtering (fig.10 : bottom panel), and used to model the respective LEP peak in the predetermined time window. Thereafter, these basic sets were regressed against the corresponding time window of each single LEP epoch. For a negative fit ($\beta i<0$) N1 and N2 are determined as the most positive peak and P2 as the most negative peak. For a positive fit ($\beta i>0$) the situation is vice versa. The peaks are calculated within a 100 ms wide time window, centred on the latency of the corresponding LEP peak model identified in the average waveform. On these regressors the peak latency and peak-to-baseline amplitude are estimated for each trial. Habituation index (HI) was calculated based on these values. Finally, average LEP properties were determined (39, 51).

3.4.3 Automated single trial analysis in the fibromyalgia group

For each subject and each stimulus site, onset-to-peak latency and peak-to-baseline amplitude of the evoked potentials (N1, N2, P2) were computed on the averaged waveform after ASTA. Peak-to-peak duration and peak-to-peak amplitude were calculated from these values. The habituation index (HI) was calculated with single trial data obtained from the ASTA as described in section 4.6.1.

3.4.4 Automated single trial analysis in the control groups

From two studies by Hatem et al (2012) and Ragé et al (2011), raw data obtained after approximate 30 laser stimulations on each examination site, were analyzed using the same pre-processing and processing methods as described above (35, 51). The recordings were performed in the Algology Laboratory (UCL) in Brussels, Belgium by S.H. (Hand-controls) or M.R. (Foot-controls).

For each subject and each stimulus site, onset-to-peak latency and peak-to-baseline amplitude of the evoked potentials (N1, N2, P2) were computed on the averaged waveform after ASTA. Peak-to-peak duration and peak-to-peak amplitude were calculated from these values.

For the present study, the habituation index (HI) was calculated as described in section 4.6.1.

3.5 Statistical analysis

(65)

The software package SPSS statistics 17.0 was used for statistical analyses. In all cases , a *p*-value below 0.05 was considered as significant.

This research used raw data obtained retrospectively from a large data basis of LEP recordings. After applying a standardized and validated protocol, the demographic characteristics, psychophysical parameters and electrophysiological features were described. These individually obtained data were compared with published control values and with values of age- and gender-matched healthy control subjects from two ancient experiments by Hatem et al (2012) and Ragé et al (2011) (35, 51).

First, data were inspected with descriptive statistics (Table 2 and 3). The central benchmarks to discuss our continuous variables were the mean value and standard deviation (SD).The age of one subject (hand, FMS) wasn't known: in this case the mean age (51 years) was used for further analysis. Descriptive statistics were not corrected for age: the impact of this factor will be discussed in topic 4.2.

The data distribution was tested with the Levene's test and by observing data histograms.

An unpaired Wilcoxon test (a.k.a. Mann-Whitney-U-test) was performed in order to ascertain whether the left - and right-hand or foot side could be viewed as two independent samples originating from the same population.

LEP amplitudes, for each stimulation site (hand and foot) separately, were submitted to an univariate analyses of co-variance (ANCOVA) between the independent group of FMS subjects and HCs, with a correcting covariate age (except for N1 foot amplitude due to loss of healthy control N1 data).

Next, the pre-assumed relationship between the different LEP amplitudes derived after hand and foot stimulation, was computed. Correlation tests were implemented to observe body side difference in the different wave-forms. The relative distributions of the N1 - and N2-P2 amplitude values at hand or foot stimulation site were first displayed with a Kernel function estimate. Kernel density estimators have the advantage to smooth out the contribution of each observed data point over a local neighbourhood of that data point (in contrast with conventional histograms which depend on the width - and end points of the bins). This non-parametric density estimator has no fixed structure and depends upon the value of its bandwidth. The most appropriate bandwidth is between 0.1 and 0.5. In the N2-P2 peak-to-peak and N1 peak-to-baseline amplitude correlation of hand vs. foot stimulation, the peak values were not corrected for age, because the relation was drawn between two measurements of the same individual.

Thereafter, the impact of the covariate age on the LEP latencies, and possible differences in LEP latencies between FMS patients and HCs, was statistically approached. The intra-individual and intra-single-trial peak correlations were calculated with an intra-class correlation coefficient (ICC). This test was used to quantify the degree to which individual units, with a fixed degree of relatedness, have the exact same value.

For correlation analyses between LEP latencies and age, the bivariate Spearman correlation test was used. This was followed by an one-way analysis of variance (ANOVA) between FMS subjects and HCs to assess differences between dependent variables, with the degree of freedom (Df) equal to 1.

Finally, correlations between the N2-P2 - and N1 peak amplitude, and between the LEP amplitudes and habituation index, were assessed with a Spearman rank correlation test.

4 Results

4.1 Descriptive statistics

85 ambulant FMS patients (26 males/ 59 females, aged 25-84 years old, mean 51 years) met all stated requirements and were considered for inclusion in this study.

No significant difference (Chi-square test) in gender was observed between the FMS and HCs group after hand or foot stimulation (Table 10 and 11).

No significant difference (Wilcoxon - / Mann-Whitney-U-test) in age was observed between the FMS and HCs group after hand or foot stimulation (Table 10 and 11).

The body side distribution turned out to be different in FMS compared to the HCs group. But, no significant difference (Wilcoxon - / Mann-Whitney-U-test) in body side stimulation was observed between the FMS and HCs group after hand or foot stimulation (Table 10 and 11), proving so the null hypothesis that these two groups come from a same population. Thus, it should be considered to interlard both sides (left and right) as one group.

The number of stimuli given, the ratio man/woman and the age (although the HCs were a bit younger) was similar between automated and visual analyses data.

The distribution of the different samples is discussed in Table 12.

Table 10 *Demographic features and LEP measurements of FMS patients and controls after laser-stimulation on the hand dorsum. Where applicable, values are given as medians ± 1 standard deviation. All values are averaged from both body sides and uncorrected for age. Results of statistical analysis between groups are reported. For the one-way ANOVA test (non-age corrected) and the Kruskal-Wallis-tests, the degree of freedom (df) was 1 (N.A. = not applicable)*

	FMS	Healthy Controls	
Laser stimulus intensity (mJ)	723 ± 117	768 ± 72	
Stimulus density (mJ/mm²)	9.3 ± 1.3	9.6 ± 0.9	
HAND		Ref. (55)	
N =	85	21	
Gender: M/F	26/59	7/14	X^2 0.06 N.S.
Age (years)	51 ± 10	47 ± 15	*U* 1075 *W* 1636 N.S.
Body side (Right/Left)	11/74	21/21	*U* 366 *W* 432 N.S
Number of stimuli	30 ± 6	30	
Detection Rate (%)	91 ± 19	100 ± 0.0	
Detection threshold (mJ/mm²)	3.4 ± 1.7	N.A.	
Pain threshold (mJ/mm²)	6.7 ± 2.1	N.A.	
Reaction Time (ms)	561 ± 246.3	R: 414 ± 84 L: 401 ± 87	
Visual N1 latency (ms)	190 ± 46.3	N.A.	
Visual N2 latency (ms)	261 ± 54.2	N.A.	
Visual P2 latency (ms)	388 ± 90.7	N.A.	
Visual N1-N2 duration (ms)	70 ± 30.8	N.A.	
Visual N2-P2 duration (ms)	128 ± 55.7	N.A.	
Visual N1 amplitude (µV)	N.A.	N.A.	
Visual N2-P2 amplitude (µV)	32.7 ± 20.7	N.A.	
ASTA N1 latency (ms)	199 ± 35.0	191 ± 18.0	X^2 2.39 N.S.
ASTA N2 latency (ms)	271 ± 56.0	248 ± 27.6	X^2 2.15 N.S.
ASTA P2 latency (ms)	383 ± 92.2	351 ± 40.2	X^2 1.12 N.S.
ASTA N1-N2 duration (ms)	80 ± 56.2	60 ± 24.4	X^2 1.34 N.S.
ASTA N2-P2 duration (ms)	112 ± 69.6	104 ± 31.9	X^2 0.04 N.S.
ASTA N1 amplitude (µV)	9.6 ± 6.9	7.8 ± 4.4	*F* 1.47 N.S.
ASTA N2-P2 amplitude (µV)	42.3 ± 27.3	26.2 ± 14.6	*F* 6.78 *p* 0.011
Habituation index	-0.11 ± 0.22	N.A.	
Non-/Abnormal-/Normal Habituaters (N =)	28/38/19	N.A.	
1e repetition mean Amp N2-P2	46.4	N.A.	
3e repetition mean Amp N2-P2	36.9	N.A.	

Table 11 *Demographic features and LEP measurements in FMS patients and controls after laser-stimulation on the foot dorsum (controls: distal leg). Where applicable, values are given as medians ± 1 standard deviation. All values are averaged from both body sides and uncorrected for age. Results of statistical analysis between groups are reported. For the one-way ANOVA test (non-age corrected) and the Kruskal-Wallis-tests, the degree of freedom (df) was 1 (N.A. = not applicable)*

	FMS	Healthy Controls	
Laser stimulus intensity (mJ)	723 ± 117	-	
Stimulus density (mJ/mm²)	9.3 ± 1.3	-	
FOOT		Ref. (35)	
N =	85	18	
Gender: M/F	26/59	12/6	X^2 3.49 N.S.
Age (years)	51 ± 10	43 ± 9	*U* 1075 *W* 1636 N.S.
Body side (Right/Left)	42/43	2/16	*U* 640 *W* 1543 N.S
Number of stimuli	34 ± 7	30	
Detection Rate (%)	83 ± 23	94	
Reaction Time (ms)	825 ± 422.8	502 ± 346	
Visual N1 latency (ms)	227 ± 54.5	207 ± 30.5	
Visual N2 latency (ms)	303 ± 62.1	272 ± 31.6	
Visual P2 latency (ms)	441 ± 83.5	432 ± 71.1	
Visual N1-N2 duration (ms)	75 ± 29.2	67 ± 30.0	
Visual N2-P2 duration (ms)	138 ± 49.4	160 ± 44.1	
Visual N1 amplitude (µV)	N.A.	3.7 ± 3.7	
Visual N2-P2 amplitude (µV)	22.7 ± 15.0	26.1 ± 13.0	
ASTA N1 latency (ms)	218 ± 51.4	N.A.	
ASTA N2 latency (ms)	277 ± 66.7	229 ± 56.9	X^2 5.41 *p* 0.02
ASTA P2 latency (ms)	425 ± 81.0	421 ± 52.2	X^2 0.00 *p* 0.982
ASTA N1-N2 duration (ms)	86 ± 66.4	N.A.	
ASTA N2-P2 duration (ms)	148 ± 71.2	192 ± 77.2	X^2 3.85 N.S.
ASTA N1 amplitude (µV)	6.5 ± 3.9	N.A.	
ASTA N2-P2 amplitude (µV)	29.0 ± 16.3	28.8 ± 10.8	*F* 0.133 N.S.
Habituation index	-0.06 ± 0.23	N.A.	
Non-/Abnormal-/Normal Habituaters (N =)	33/42/10	N.A.	
1e repetition mean Amp N2-P2	31.4	N.A.	
3e repetition mean Amp N2-P2	27.4	N.A.	

Table 12 *Results of the Kolmogorov-Smirnov test to determine if the observed distribution of each sample corresponds to a theoretical normal distribution (2-tailed Sig.)*

HAND		**Latency**		**Duration**		**Amplitude**	
FMS	**N1**	**N2**	**P2**	**N1N2**	**N2-P2**	**N1**	**N2-P2**
K-S Z	1.091	1.789	1.328	2.097	1.427	1.319	1.503
P	0.185	0.003	0.059	0.000	0.034	0.062	0.022
Distr	Normal	Not normal	Boundary	Not normal	Boundary	Boundary	Not normal
HCs	**N1**	**N2**	**P2**	**N1N2**	**N2-P2**	**N1**	**N2-P2**
K-S Z	0.660	0.465	0.756	0.925	0.557	0.752	0.666
p	0.776	0.982	0.618	0.359	0.915	0.624	0.767
Distr	Normal	Normal	Normal	Normal	Normal	Normal	Normal

FOOT	**Latency**		**Duration**		**Amplitude**
FMS		**N2**	**P2**	**N2-P2**	**N2-P2**
K-S Z		0.666	0.657	1.178	1.323
P		0.767	0.781	0.125	0.060
Distr		Normal	Normal	Normal	Boundary
HCs		**N2**	**P2**	**N2-P2**	**N2-P2**
K-S Z		0.819	0.676	0.653	0.502
p		0.514	0.750	0.787	0.963
Distr		Normal	Normal	Normal	Normal

Peak duration is defined as the side-to-side difference in time of the begin in one deflection and the end of cosmic deflections (see section 2.2.8.5).

4.2 Laser-evoked potential amplitudes in fibromyalgia and healthy controls

4.2.1 N2-P2 peak-to-peak amplitude: hand

4.2.1.1 Equality of Variance

Equality of variance between the FMS (σ^2 746.1) and HCs (σ^2 213.0) group was evaluated by means of a Levene's test. No significance difference was found, meaning the variance of the dependent variable (N2-P2 amplitude) is assumed equal across groups.

4.2.1.2 Kernel distributions of FMS vs. control (hand)

The distribution of the N2-P2 amplitudes was examined by using the Kernel method. This method indicated that the distribution of both groups was skewed (and not normal). (Fig.11)

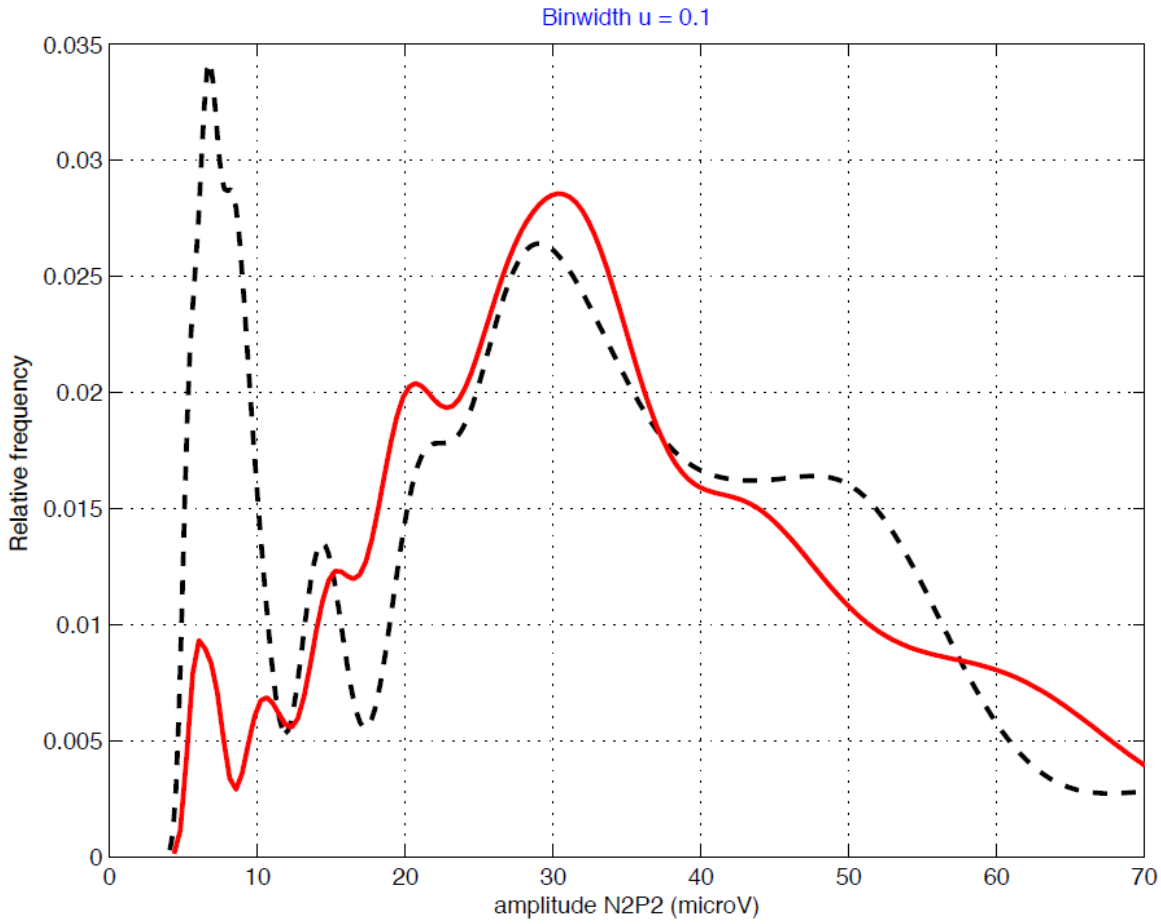


Fig.11 *A kernel function estimate (bin-width of 0.1) with the N2-P2 peak-to-peak amplitude obtained in FMS patients (red line) and HCs (black dots) after hand laser-stimulation*

4.2.1.3 Linear regression

The influence of age on the N2-P2 amplitudes was examined by plotting a linear regression (Figure 12). This analysis indicated that there is a linear relationship between age and N2-P2 amplitude in both groups.

FMS:	Y = -0.729 . X + 75.18	R^2 = 0.158
HCs:	Y = -0.6152 . X + 55.06	R^2 = 0.3925

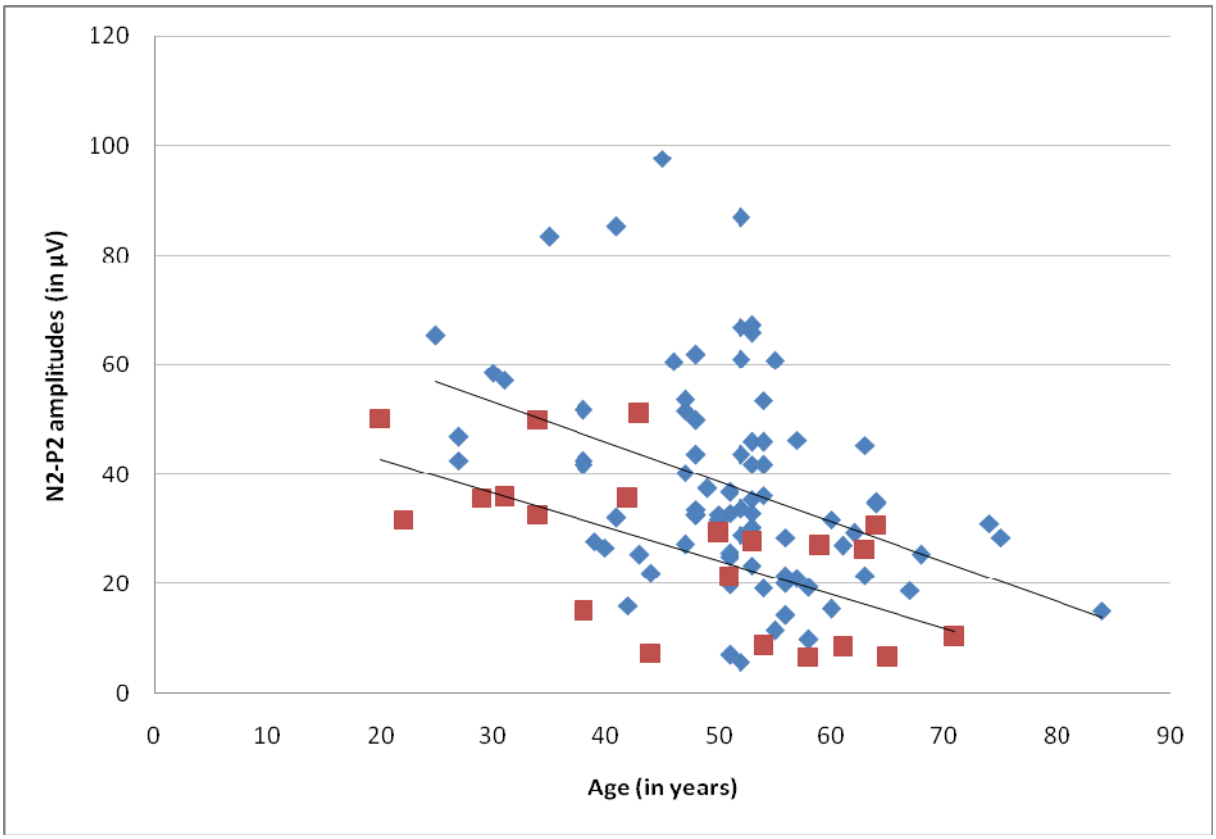


Fig. 12 *N2-P2 peak-to-peak amplitude values obtained after laser-stimulation on the hand dorsum, as a function of the age in FMS patients (blue dots) and healthy controls (red dots). It can be observed clearly that N2-P2 amplitude decreases with age. Interestingly, LEP amplitudes in FMS patients also decrease with age with a greater slope than healthy controls. Four outliers were rejected for analysis*

Thus, a correction for age should be applied in order to interpret correctly the N2-P2 amplitudes in both groups. The values corrected for age can be found in the Table underneath.

4.2.1.4 Age corrected values

Table 13 *Descriptive statistics: N2-P2 peak-to-peak amplitudes of FMS and HCs , expressed in means ± SD, without and with adjustment for age as a covariate*

	Without age correction	**With age correction**
FMS	42.3 µV ± 27.3	42.9 µV ± 23.2
HCs	26.2 µV ± 14.6	23.5 µV + 23.6

Without adjusting for the age as a covariate the N2-P2 peak-to-peak amplitudes were as described in Table 13. For the reasons explained above , these values were corrected for age and the new estimated N2-P2 peak-to-peak amplitude means ± standard deviation after hand stimulation are also displayed in Table 13.

After padding of means: F = 45.08, P < 0.001.

4.2.1.5 Analysis of co-variance

The homogeneity of the regression was tested by means of a Levene's test. No significant difference could be demonstrated between both variances, thus they can be considered equal.

Table 14 *ANCOVA statistics, with N2-P2 peak-to-peak amplitude as dependent variable and age as a covariate, between FMS subjects (N = 85) and healthy controls (N = 21) after laser-stimulation on the hand dorsum*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	14587,265[a]	2	7293,632	13,248	,000	,205
Intercept	30489,058	1	30489,058	55,378	,000	,350
Age	10224,383	1	10224,383	18,571	,000	,153
Groups	6248,468	1	6248,468	11,349	,001	,099
Error	56707,667	103	550,560			
Total	233219,878	106				
Corrected Total	71294,932	105				

a. R^2 = .205 (Adjusted R^2 = .189)

The ANalysis of CO-VAriance (ANCOVA) (with a one DF and cofactor age) yielded the following results: F = 11.349, P = 0.001. (Table 14) A very high significant difference was observed between the N2-P2 amplitudes of FMS patients and their age-matched controls, even when using age as a covariate. This means that age is an important confounding factor when interpreting the LEP values of FMS patients. This finding was confirmed when plotting the LEP amplitude in function of age (Fig.12). Thus, the original amplitude values obtained with ASTA were corrected for age.

In brief, LEP results in FMS should be corrected for the patients' age. This correction increases the difference observed between FMS patients and healthy controls.

4.2.2 N2-P2 peak-to-peak amplitude: foot

4.2.2.1 Equality of Variance

Equality of variance between the FMS (σ^2 265.5) and HCs (σ^2 299.3) group was evaluated by means of a Levene's test. No significance difference was found, meaning the variance of the dependent variable (N2-P2 amplitude) is assumed equal across groups.

4.2.2.2 Kernel distributions of FMS vs. control (foot)

The distribution of the N2-P2 amplitudes was examined by using the Kernel method. This method indicated that the distribution of both groups was skewed (and not normal). (fig.13)

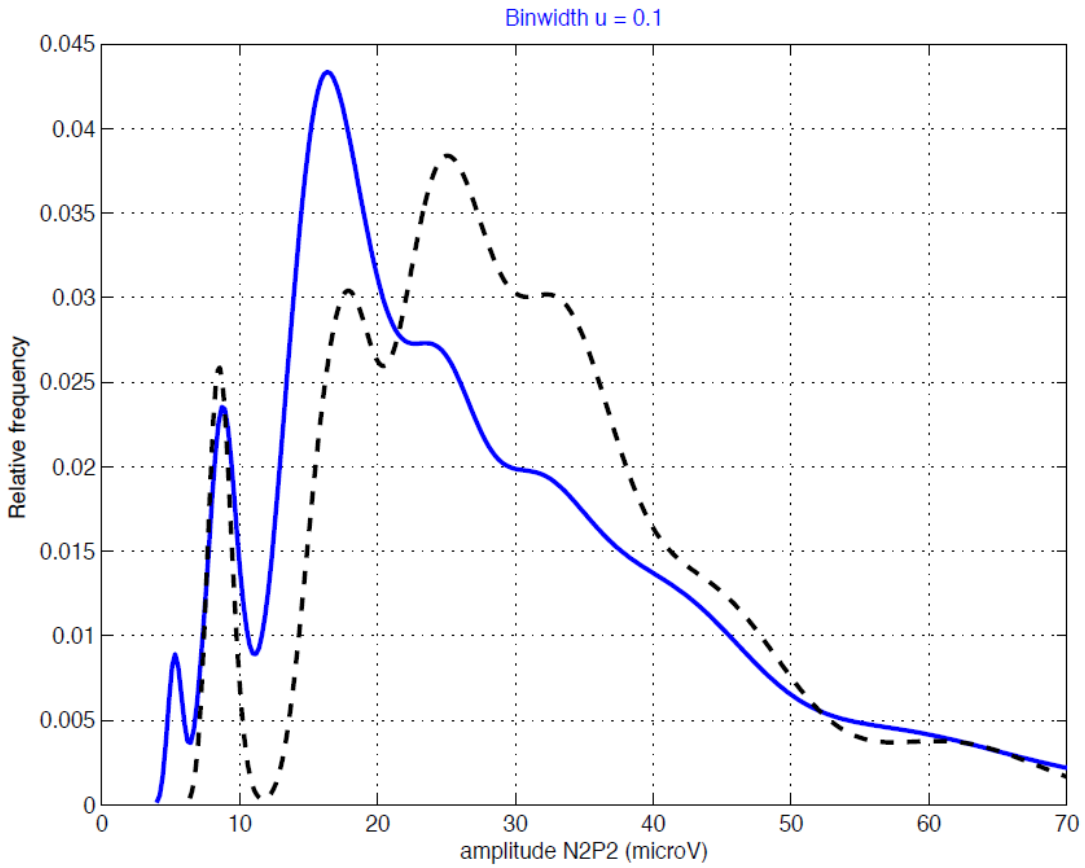


Fig.13 *A kernel function estimate (bin-width of 0.1) with the N2-P2 peak-to-peak amplitude obtained in FMS patients (blue line) and HCs (black dots) after foot laser-stimulation*

4.2.2.3 Linear regression

The influence of age on the N2-P2 amplitudes was examined by plotting a linear regression (Figure 14). This analysis indicated that there is no linear relationship between age and N2-P2 amplitude in both groups.

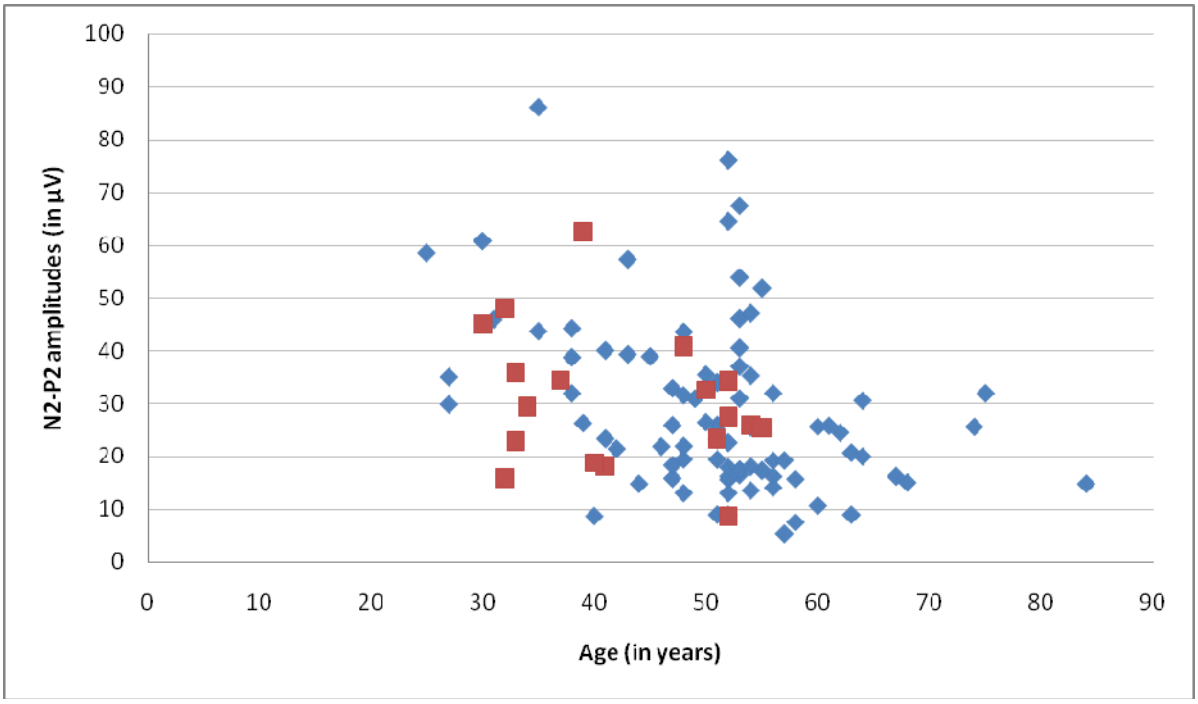


Fig. 14 *N2-P2 peak-to-peak amplitude values obtained after laser-stimulation on the foot dorsum, as a function of the age in FMS patients (blue dots) and healthy controls (red dots). It can be observed that N2-P2 amplitude decreases with age*

4.2.2.4 Analysis of co-variance

The homogeneity of the regression was tested by means of a Levene's test. No significant difference could be demonstrated between both variances, thus they can be considered equal (Table 15).

Table 15 *ANCOVA statistics, with N2-P2 peak-to-peak amplitude as dependent variable and age as a covariate, between FMS subjects (N = 85) and healthy controls (N = 18) after laser-stimulation on the foot dorsum*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3567,616[a]	2	1783,808	8,266	,000	,143
Intercept	14168,950	1	14168,950	65,658	,000	,399
Age	3534,511	1	3534,511	16,379	,000	,142
Groups	158,703	1	158,703	,735	,393	,007
Error	21364,023	99	215,798			
Total	112304,345	102				
Corrected Total	24931,638	101				

a. R^2 = .143 (Adjusted R^2 = .126)

An ANCOVA (with age as a covariate) was performed to compare N2-P2 amplitudes between FMS and healthy subjects. This test (with the DF equals 1) had the following results: F = 0.735, P = 0.393. (Table 15) No significant difference was observed between the N2-P2 amplitudes of FMS patients and their age-matched controls, even after correction for age (fig. 14).

4.2.3 N1 peak-to-baseline amplitude: hand

4.2.3.1 Equality of variance

Equality of variance between the FMS (σ^2 47.6) and HCs (σ^2 19.6) group was evaluated by means of a Levene's test. No significance difference was found, meaning the variance of the dependent variable (N1 amplitude) is assumed equal across groups.

4.2.3.2 Kernel distributions of FMS vs. control (hand)

The distribution of the N1 amplitudes was examined by using the Kernel method. This method indicated that the distribution of both groups was skewed (and not normal).

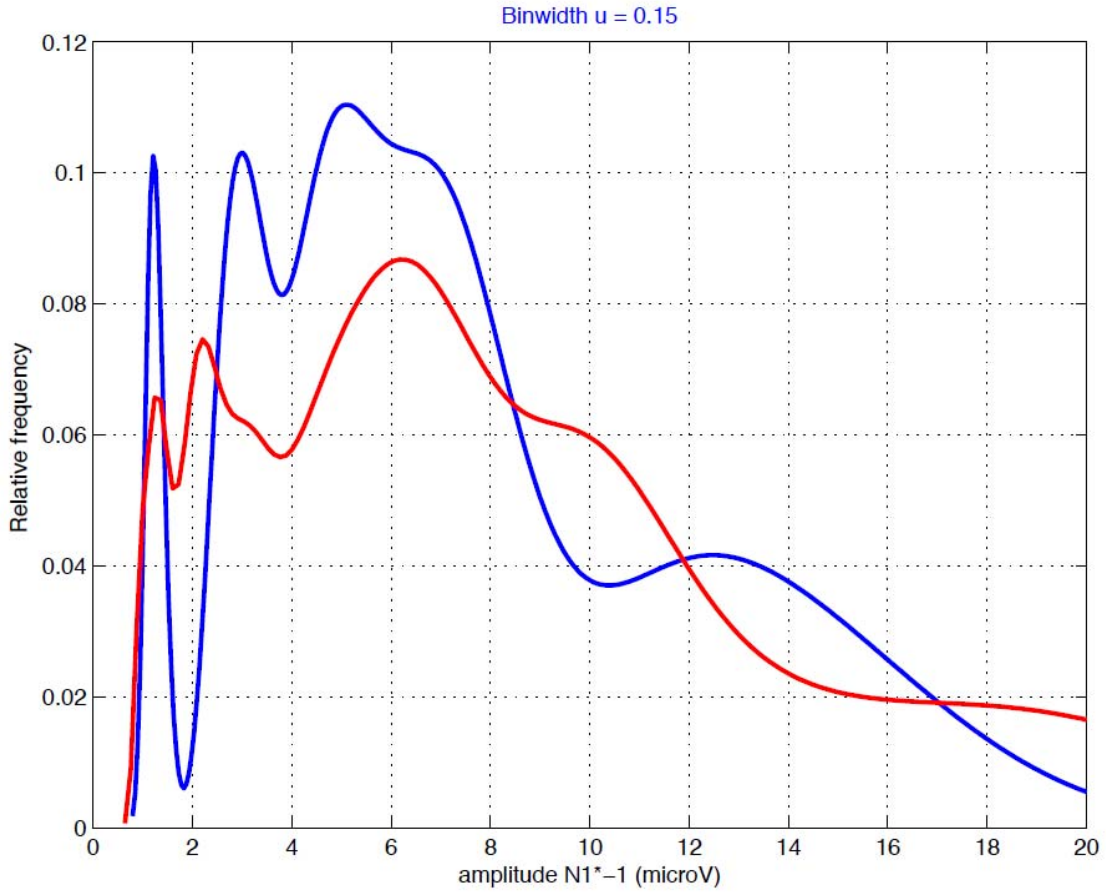


Fig.15 *A kernel function estimate (bin-width of 0.15) with the N2-P2 peak-to-peak amplitude obtained in FMS patients (red line) and HCs (blue line) after hand laser-stimulation*

4.2.3.3 Linear regression

The influence of age on the N1 amplitudes was examined by using a linear regression method (Figure 16). It showed that both in FMS patients as in healthy controls, N1 amplitude decreased with increasing age. Possible linear relation between age and N1 amplitude in both groups can be described by:

FMS	Y = - 0.203 . X + 19.86	R^2 = 0.116
HCs	Y = - 0.126 . X + 13.76	R^2 = 0.1786

Too many missing values in the foot data impeded a reliable analysis of this peak.

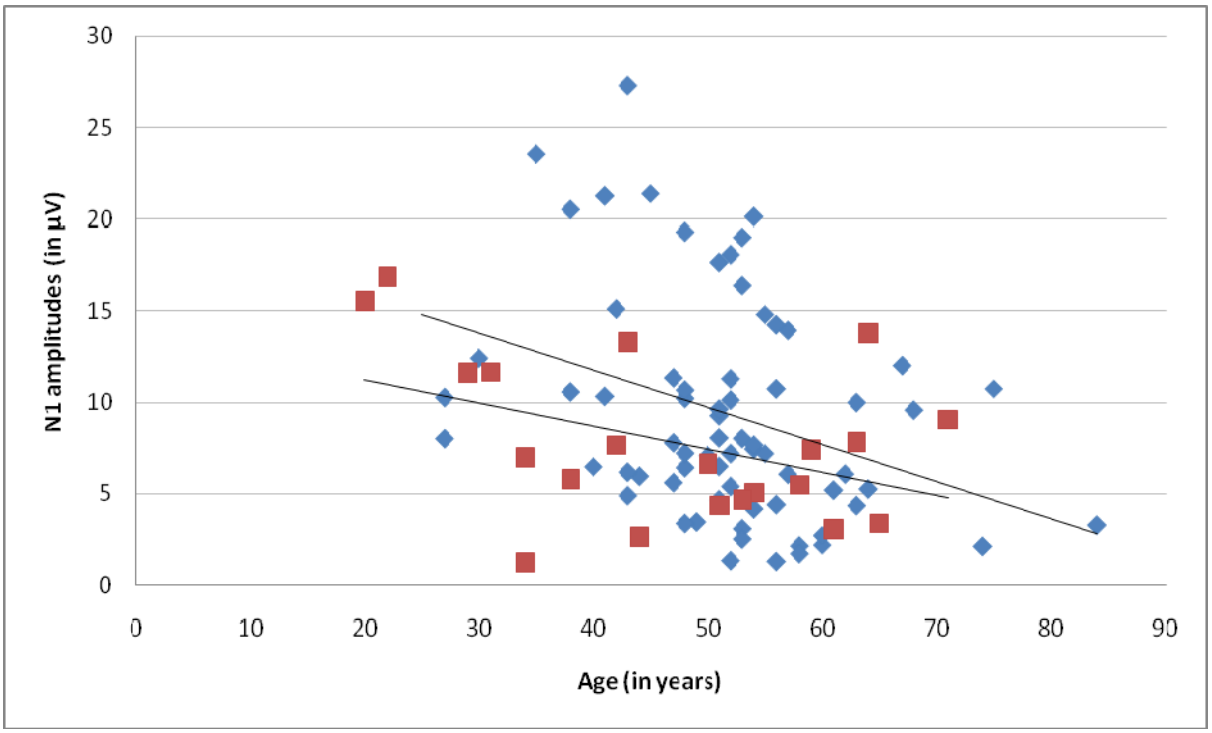


Fig. 16 *N1 peak-to-baseline amplitude values obtained after laser-stimulation on the hand, in function of the age of the FMS - (blue dots) and control (red dots) patients. It can be observed that N1 amplitude decreases with age. Interestingly, LEP amplitudes in FMS patients also decrease with age with (initially) a greater slope than healthy controls*

Thus, a correction for age should be applied in order to interpret correctly the N1 amplitudes in both groups. The values corrected for age can be found in the Table underneath.

4.2.3.4 Age corrected values

Table 16 *Descriptive statistics: N1 peak-to-baseline amplitudes of FMS and HCs , expressed in means ± SD, without and with adjustment for age as a covariate*

	Without age correction	**With age correction**
FMS	9.8 µV ± 6.9	10.0 µV ± 6.0
HCs	7.8 µV ± 4.4	7.1 µV ± 6.1

Without adjusting for the age as a covariate the N1 peak-to-baseline amplitudes were as described in Table 16. For the reasons explained above , these values were corrected for age and the new estimated N1 peak-to-baseline amplitude means ± standard deviation after hand stimulation are also displayed in Table 16.

After padding of means: F = 11.89, P = 0.001.

4.2.3.5 Analysis of co-variance

The homogeneity of the regression was tested by means of a Levene's test. No significant difference could be demonstrated between both variances, thus they can be considered equal.

Table 17 *ANCOVA statistics, with N1 peak-to-baseline amplitude as dependent variable and age as a covariate, between FMS subjects (N = 70) and healthy controls (N = 21) after laser-stimulation on the hand dorsum*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	574,068[a]	2	287,034	7,988	,001	,154
Intercept	1635,684	1	1635,684	45,522	,000	,341
Age	513,425	1	513,425	14,289	,000	,140
Groups	131,369	1	131,369	3,656	,059	,040
Error	3161,988	88	35,932			
Total	11657,291	91				
Corrected Total	3736,056	90				

a. R^2 = .154 (Adjusted R^2 = .134)

An ANCOVA (with age as a covariate) was performed to compare N1peak amplitudes between FMS and healthy subjects. This test (with a one degree of freedom) had the following results: F = 3.656, P = 0.059. (Table 17) A trend towards a significant difference was observed between the N1 amplitude of FMS patients and controls, after correction for age (fig. 17).This means that age is probably a confounding factor when interpreting the LEP values of FMS patients. This finding was confirmed when plotting the LEP amplitude in function of age (Fig.16). Thus, the original amplitude values obtained with ASTA were corrected for age.

In brief, LEP results in FMS should be corrected for the patients' age. This correction increases the difference observed between FMS patients and healthy controls.

4.3 Laser-evoked potential latencies in fibromyalgia and healthy controls

Two questions must be answered:

- Are latencies of LEP components related to age?
- Are N1 and N2-P2 latencies of FMS subjects different compared to those of HCs?

No significant differences were observed between FMS patients and healthy controls concerning the latencies of the N1, N2 or P2 peaks.

Results of non-parametrical ANOVA (Kruskal Wallis) are described in Table 18.

Table 18 *Results of a non-parametrical ANOVA (Kruskal Wallis) observed between FMS patients and healthy controls concerning the latencies of the N1, N2 or P2 peaks*

	N_{FMS}	N_{Contr}	χ^2	df	*p*	*Sign.*
N1 Latency	70	21	2.387	*1*	0.122	N.S.
N2 Latency	85	21	2.150	*1*	0.143	N.S.
P2 Latency	85	21	1.120	*1*	0.290	N.S.

No significant correlation (Spearman rank correlation) was observed between LEP latencies and age (Fig.17 and 18).

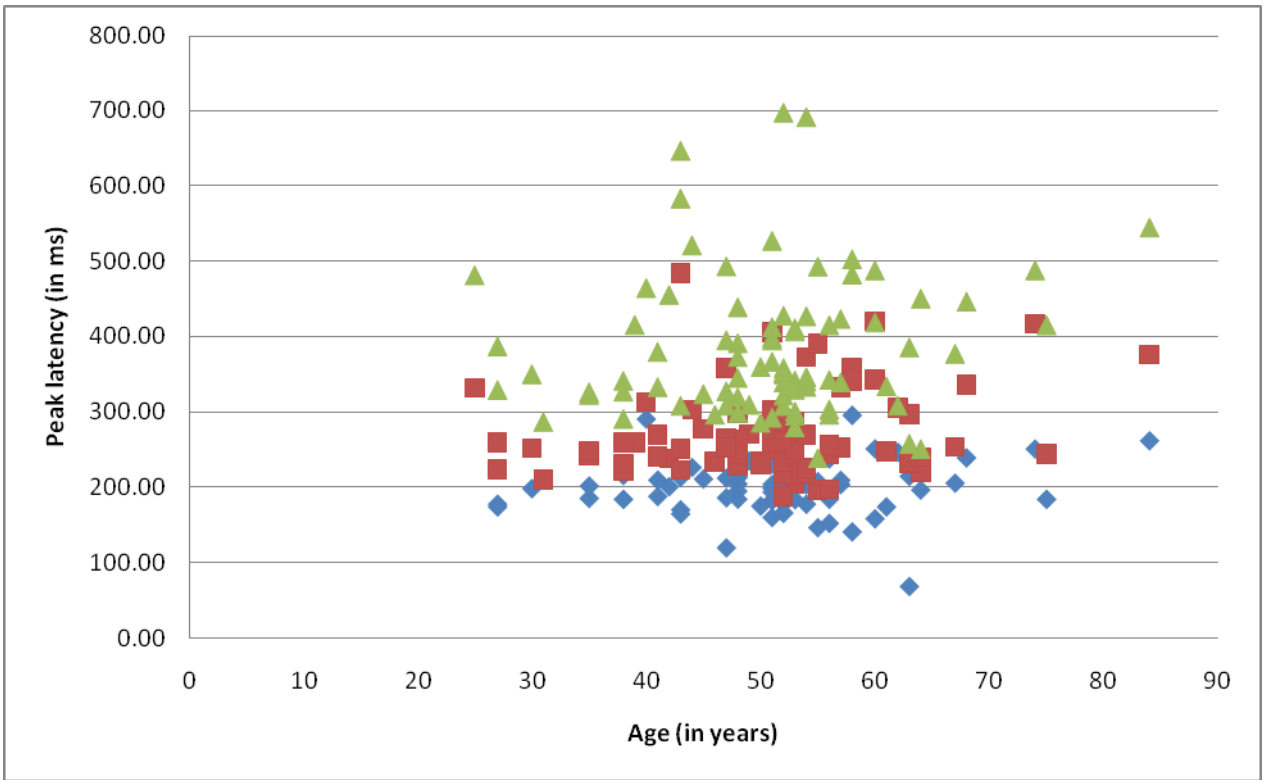


Fig. 17 *Age in function of N1 - (blue dots), N2 - (red dots) and P2 (green dots) hand LEP latency in FMS patients*

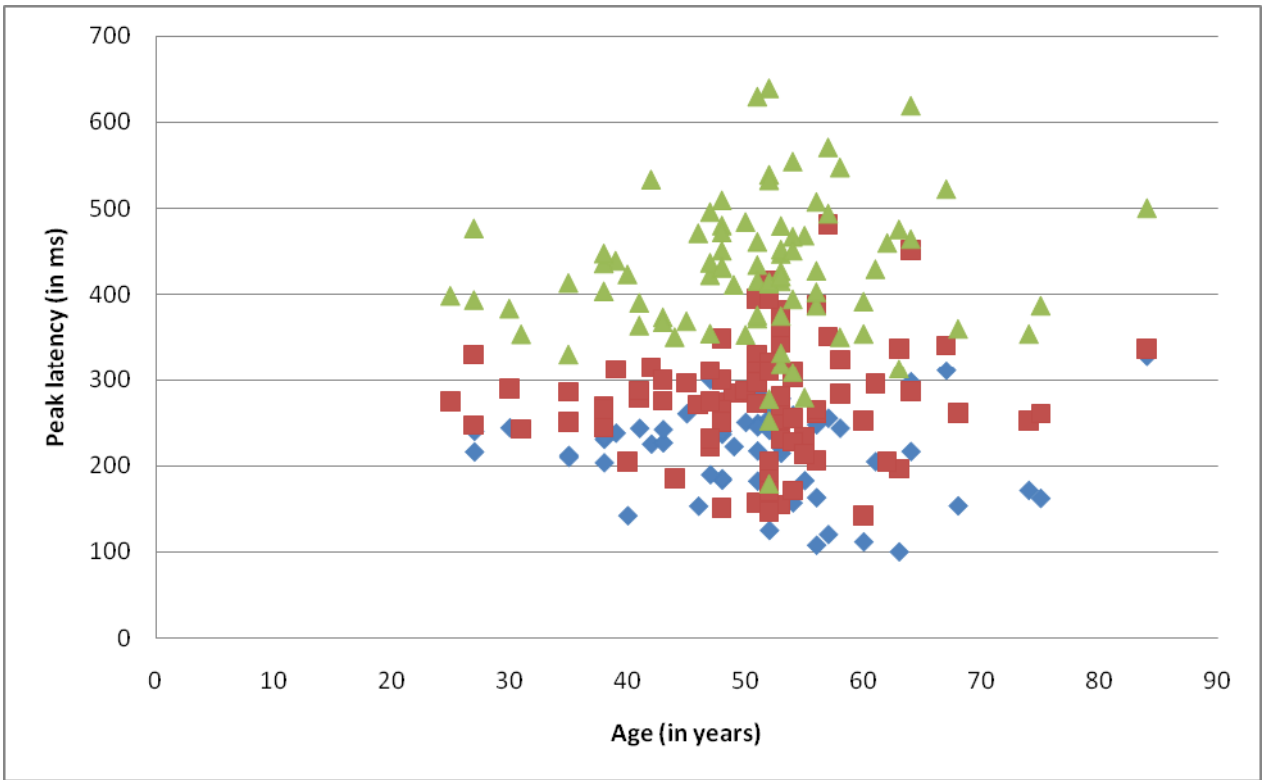


Fig. 18 *Age in function of N1 - (blue dots), N2 - (red dots) and P2 (green dots) peak latency values obtained after laser-stimulation on the foot dorsum in FMS patients*

4.4 In fibromyalgia: is there a relationship between laser-evoked potentials elicited from the hand or from the foot?

4.4.1 N2-P2 peak-to-peak amplitude: hand vs. foot

4.4.1.1 Kernel distributions of hand vs. foot stimulation (FMS)

The frequency distribution of N2-P2 amplitudes of hand and foot (84 FMS patients) were plotted with a Kernel function (Figure 19).The area under the curve (AUC) indicates the absolute number of subjects located underneath that deflection This Kernel function estimate (fig. 19), with a bandwidth of 0.1, showed that high hand N2-P2 amplitudes were more frequent than high foot N2-P2 amplitudes.

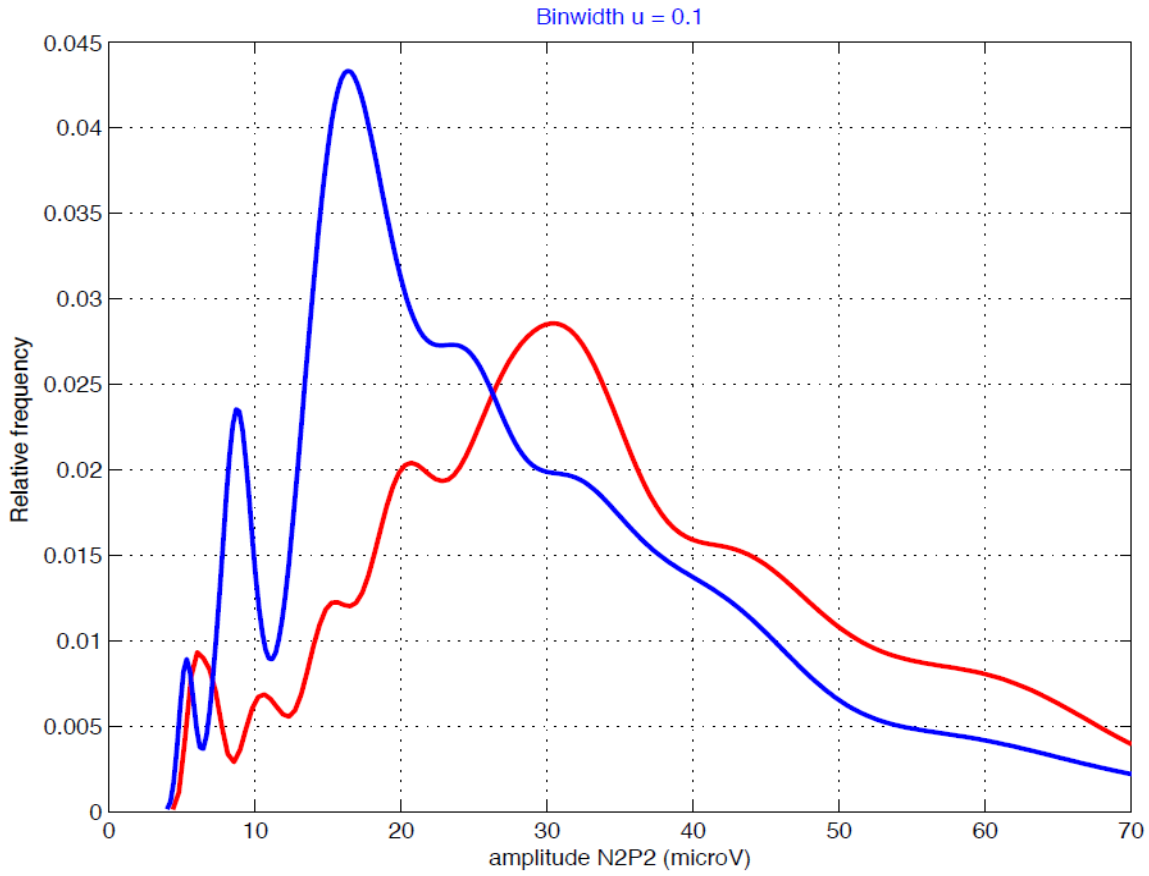


Fig. 19 *A kernel function estimate (bin-width of 0.1) with parameters N2-P2 peak-to-peak amplitude obtained after hand - (red line) and foot laser-stimulation (blue line) in FMS patients*

4.4.1.2 Linear regression

Then, the direct relationship between hand N2-P2 amplitude and foot N2-P2 amplitude (within each FMS patient) was examined by plotting the linear regression between both parameters (Figure 20). A significant difference ($F_{1,84}$ = 35.198, P < 0.0001) was observed between hand and foot N2-P2 amplitudes, as documented by a comparison with an ANOVA between both groups. There was a linear relationship between hand and foot N2-P2 amplitudes described by the equation: Y = 0.684 . X and R^2 = 0.50. This means that stimulation of the foot produced an N2-P2 of 70% of the amplitude than that obtained by stimulating the hand.

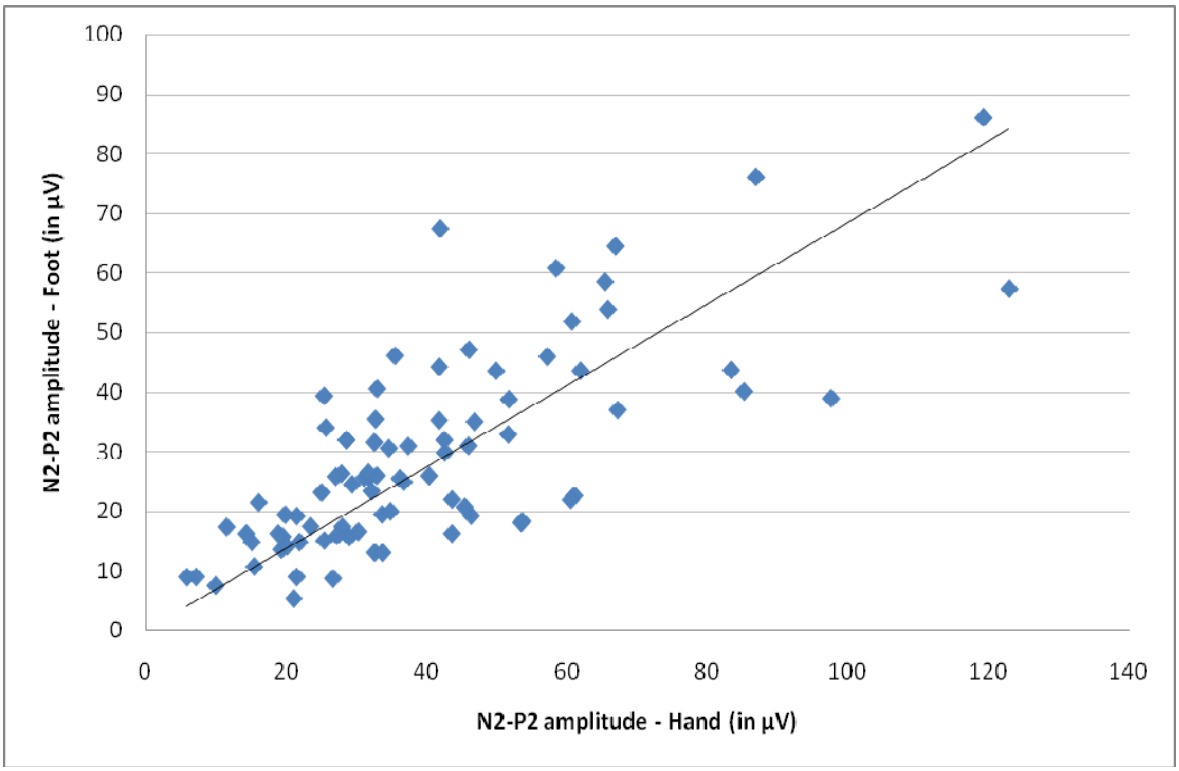


Fig. 20 *N2-P2 peak-to-peak amplitude values obtained after laser-stimulation on the foot in function of the values obtained on the hand dorsum of 84 FMS patients*

4.4.2 N1 peak-to-baseline amplitude : hand vs. foot

4.4.2.1 Kernel distributions of hand vs. foot stimulation (FMS)

The frequency distribution of N1 peak amplitude of hand and foot (50 FMS patients) were plotted with a Kernel function (Figure 21). The area under the curve (AUC) indicates the absolute number of subjects located underneath that deflection This Kernel function estimate (fig. 21), with a bandwidth of 0.15 (optimally smoothed), showed that high hand N1 amplitudes were more frequent than high foot N1 amplitudes.

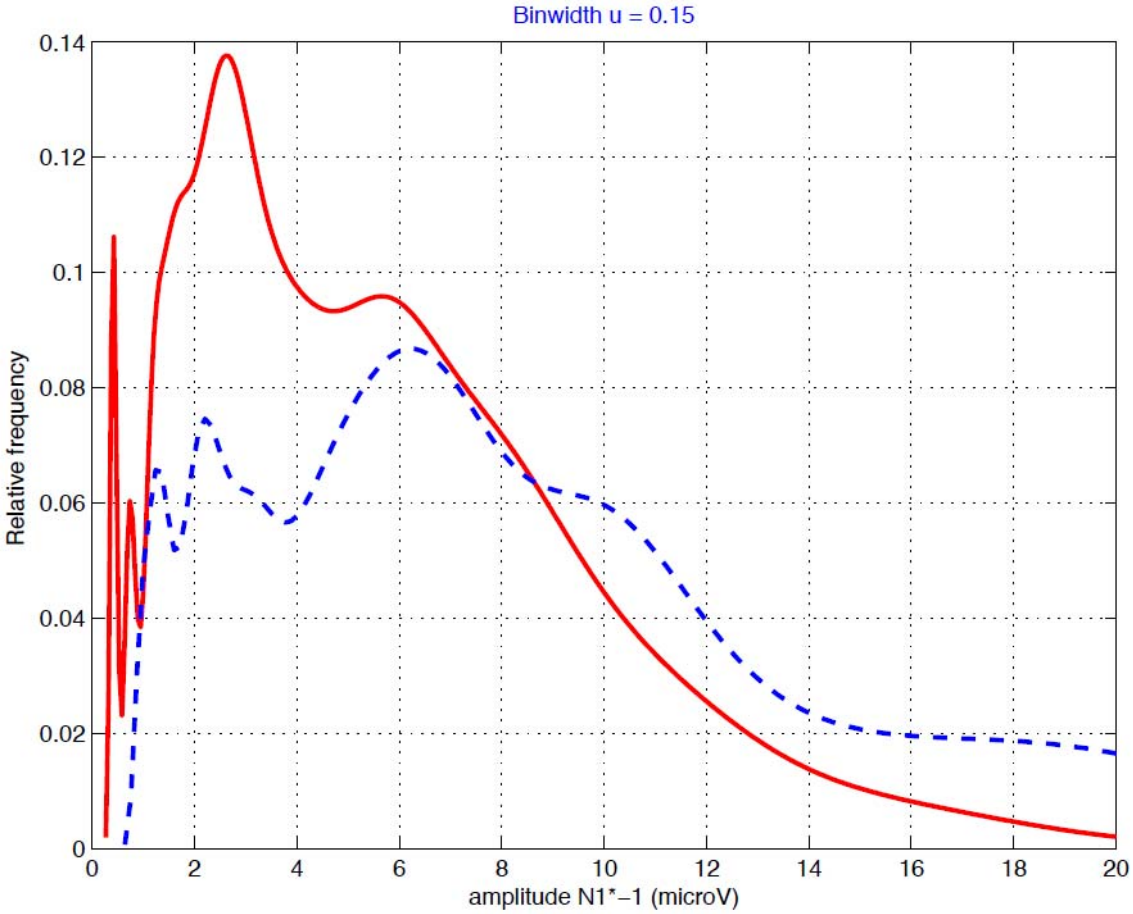


Fig. 21 *A kernel function estimate (bin-width of 0.15) with parameters N1 peak-to-baseline amplitude obtained after hand - (blue dots) and foot laser-stimulation (red line) in FMS patients*

4.4.2.2 Linear regression

Then, the direct relationship between hand N1 amplitude and foot N1 amplitude (within each FMS patient) was examined by plotting the linear regression between both parameters (Figure 22). Only 50 patients were included for this analysis, as N1 peaks could not be observed concomitantly in hand and/or foot in 35 patients. A significant difference ($F_{1,50}$ = 10.246, P = 0.002) was observed between hand and foot N1 amplitudes, as documented by a comparison with an ANOVA between both groups. There was a linear relationship between hand and foot N1 amplitudes described by the equation: Y = 0.51 . X and R^2 = 0.36. This means that stimulation of the foot produced an N1 of about half the amplitude than that obtained by stimulating the hand.

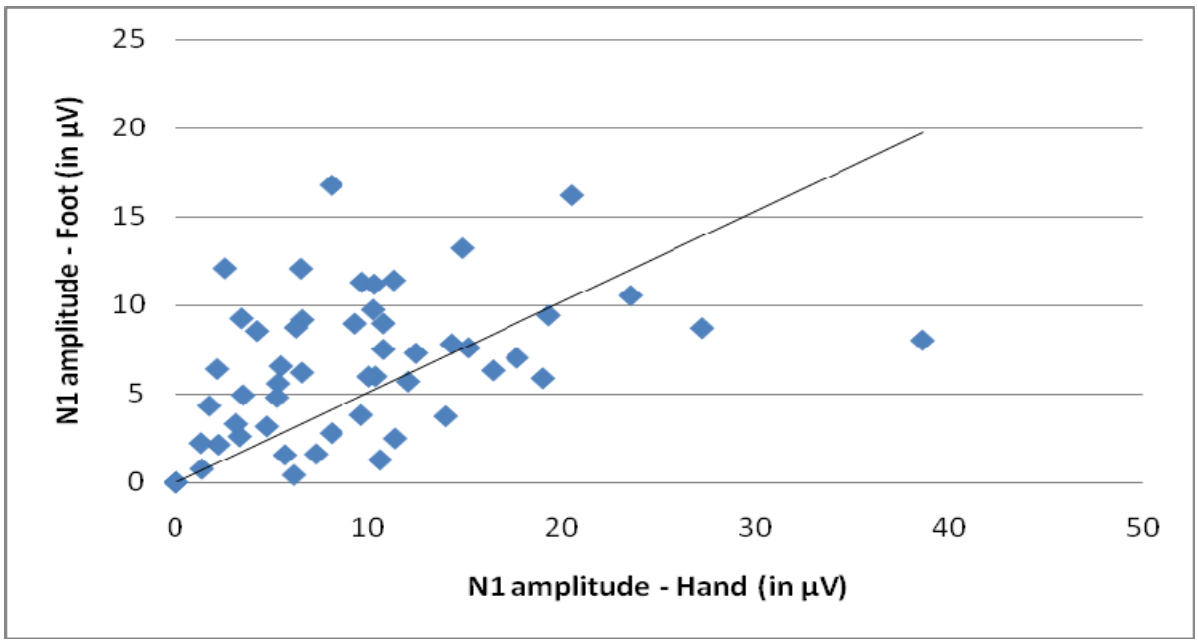


Fig. 22 *N1 peak-to-baseline amplitude values obtained after laser-stimulation on the foot in function of the values obtained on the hand dorsum of 50 FMS patients*

4.5 In fibromyalgia: is the N2-P2 amplitude dependent on the N1 peak?

To examine the relationship between N1 peak amplitude and N2-P2 peak amplitude, a linear regression was performed. As shown in fig. 23 and 24, the N2-P2 amplitude (automatic analysis) increased linearly and significantly with the amplitude of the N1 wave: both for hand LEP (*F* 59.584; *p* < 0.001) and foot LEP (*F* 6.07; *p* 0.017). N2-P2 and N1 peaks were compared by means of ANOVA . The N2-P2 is significantly depending on the N1, after the same stimulation at hand (R^2 = 0.461) or foot (R^2 = 0.097). The N2-P2 amplitude is on average 2.6 times larger than the N1 amplitude with an offset of 13.2 µV (N2-P2 = 13.2 + 2.63 * N1), after laser-stimulation of the hand dorsum, and, 1.4 times larger than the N1 amplitude with an offset of 21.9 µV (N2-P2 = 21.87 + 1.37 * N1), after laser-stimulation of the foot dorsum.

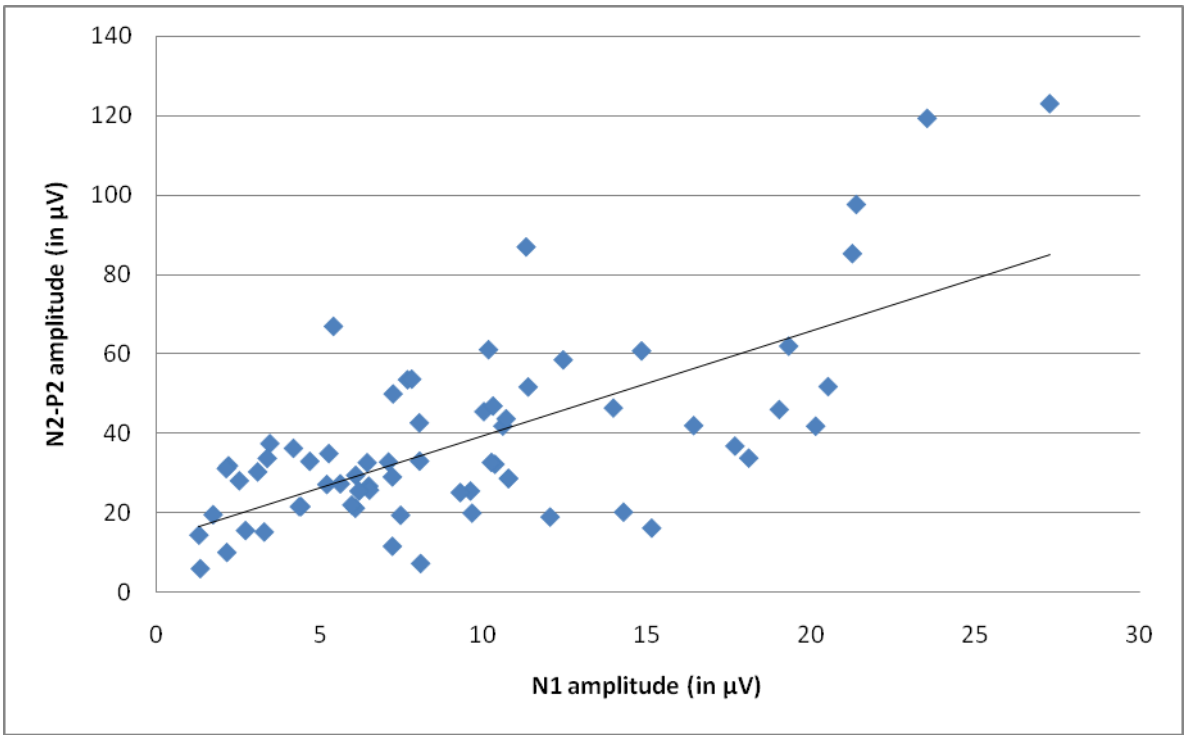


Fig. 23 *N2-P2 peak-to-peak amplitude is plotted as a function of N1 peak-to-baseline amplitude in FMS patients (Hand). One outlier was rejected for analysis*

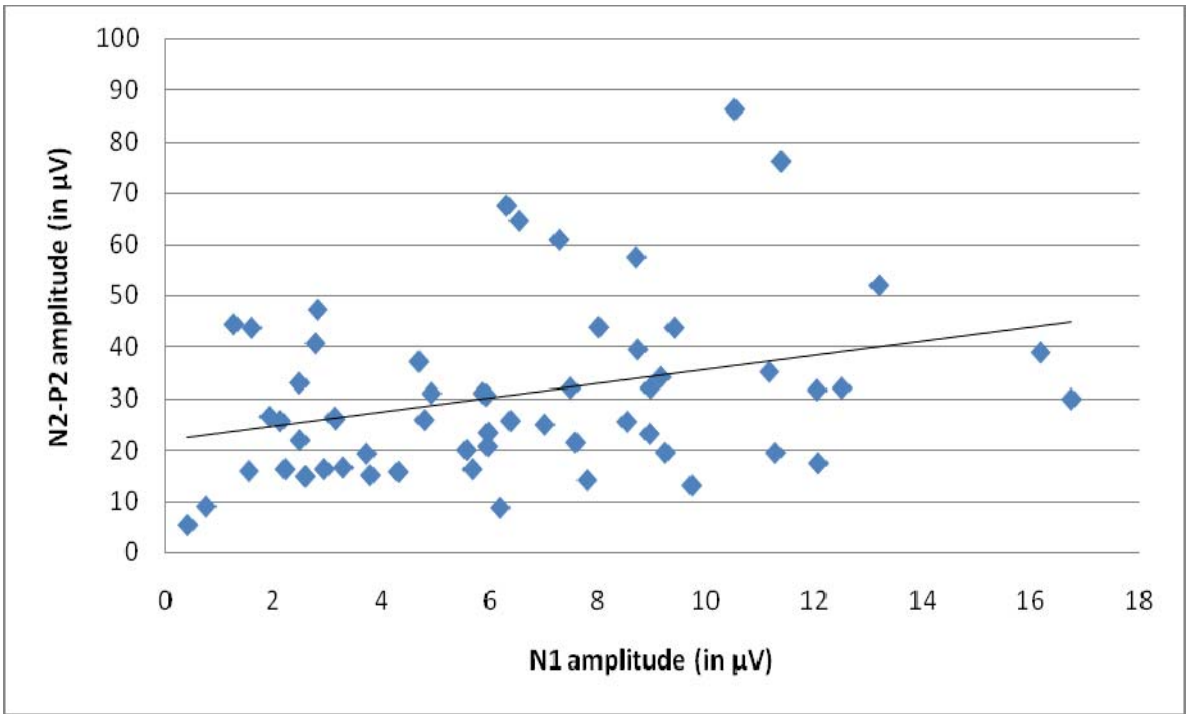


Fig. 24 *N2-P2 peak-to-peak amplitude is plotted as a function of N1 peak-to-baseline amplitude in FMS patients (Foot)*

4.6 Habituation index

4.6.1 Descriptive statistics

Peak *habituation index* (HI) was assessed by following equation:

$$HI = \frac{\text{MeanAmp}'\text{last10}' - \text{MeanAmp}'\text{first10}'}{\text{MeanAmp}'\text{first10}'}$$

In the numerator, the difference was calculated between the final averaged amplitude minus the initial mean amplitude. In the denominator we set the original, not yet degraded by habituation, averaged amplitude. This ratio shows us the relative decrease due to the habituation phenomenon.

In healthy controls, the habituation index usually is around -0.30 which means that a mean decrease of 30% of the LEP amplitude as observed over time (17, 66). Unfortunately, at present, only the HI of FMS patients was calculated and the HI of this healthy controls sample is still under calculation.

In our FMS groups (hand and foot), the HI presented both a normal, nearly Gaussian distribution, determined by a Kolmogorov-Smirnov test.

In the present sample of FMS the HI on the hand was around -0.10 (mean ± SD : -0.11 ± 0.22) and on the foot higher (mean ± SD : -0.06 ± 0.23); thus, the habituation was more reduced after foot stimulation, compared to peak recordings after hand stimulation, and in both cases noticeable deviating from the norm.

4.6.2 Relationship between HI and demographic / clinical factors

4.6.2.1 Relationship between age and habituation index

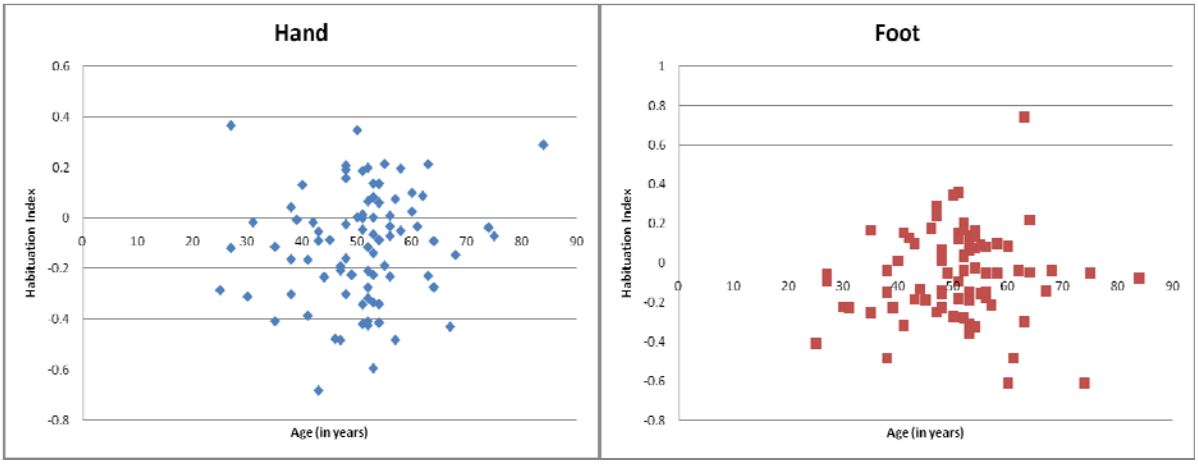


Fig. 25 *HI calculated on N2-P2 amplitude hand - (blue dots) and foot (red dots) LEP amplitude in function of age (FMS patients)*

No significant linear (Pearson correlation test) or rank (Spearman correlation test) correlation was observed between HI and age (Fig.21), except the foot HI showed solely a positive Spearman correlation (meaning a curvilinear relation, probably parabolic-like) between the HI and age (r 0.04 r_s 0.002 $p > 0.975$). (fig.25)

4.6.2.2 Relationship between gender and habituation index

No significant correlation (Spearman correlation test) was observed between HI and gender.

4.6.3 Habituation Index: hand vs. foot

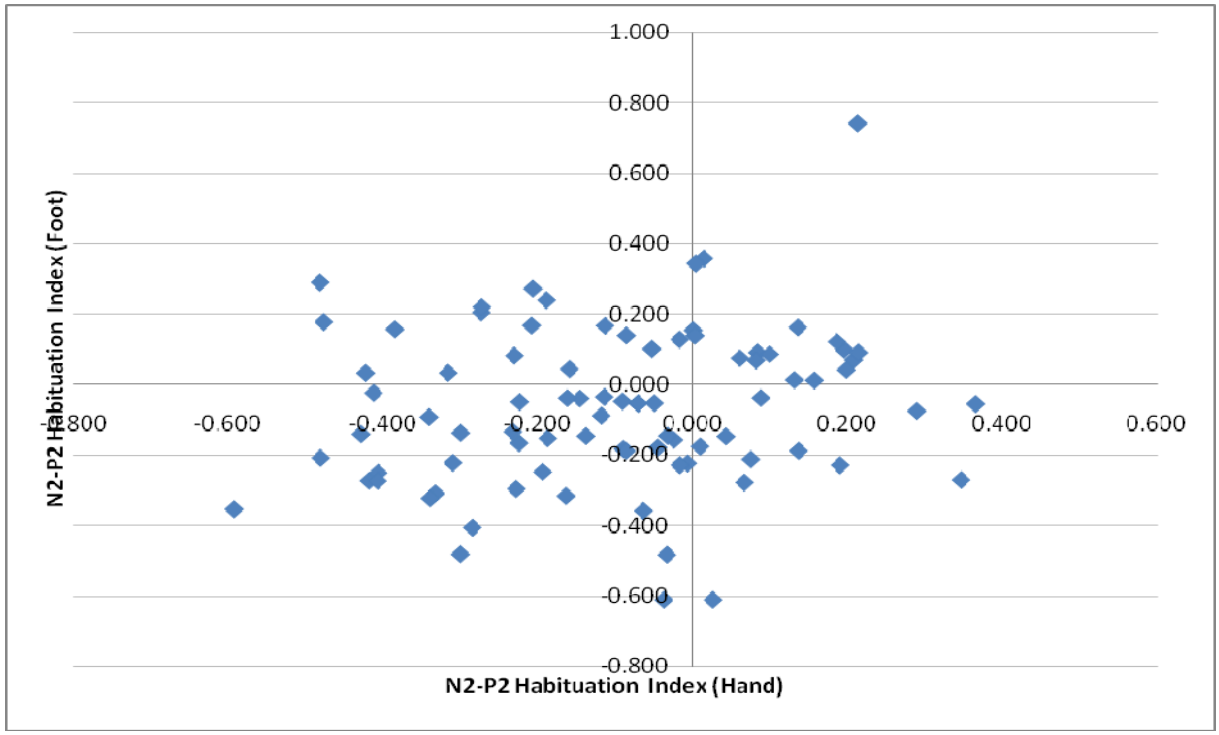


Fig. 26 *N2-P2 amplitude determined HI values of 70 FMS patients obtained after laser-stimulating the foot, compared to those after laser-stimulating the hand*

15 patients were excluded because of the absence of the N1 hand peak.

No significant relationship was observed between the N2-P2 amplitude determined HI values obtained after laser-stimulating the foot vs. hand. (fig. 26)

4.6.4 Relationship between the N1 peak and the Habituation Index

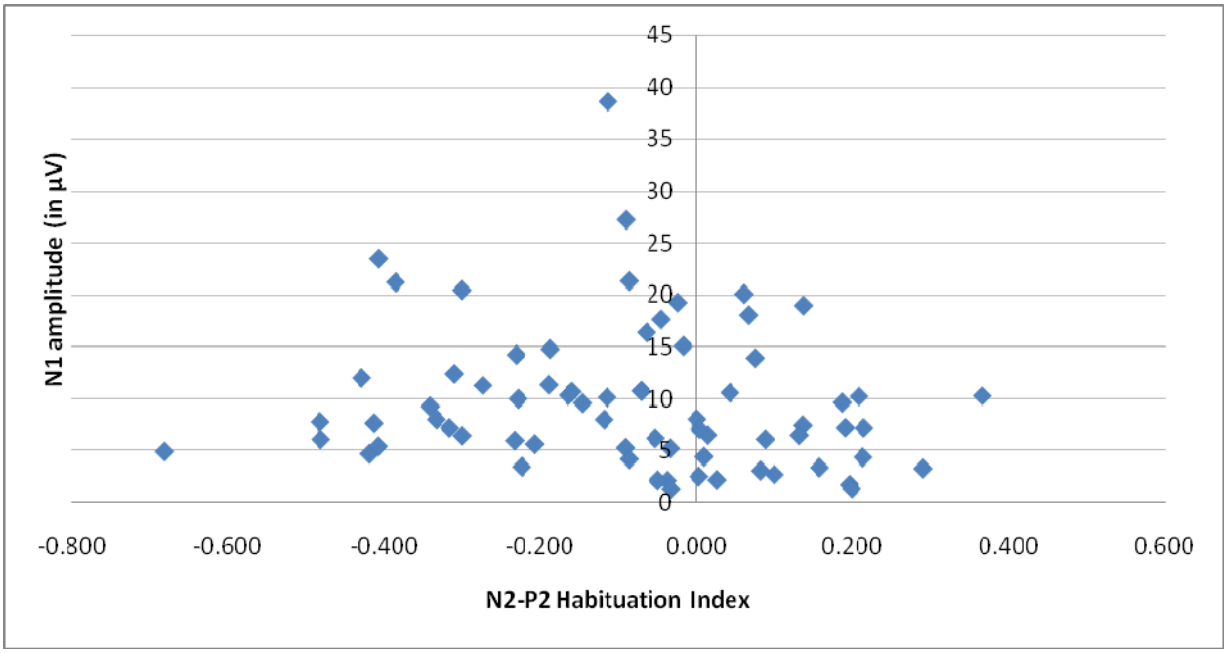


Fig. 27 *N1 peak-to-baseline amplitude values obtained after laser-stimulation on the hand in function of the calculated habituation index, based on first and third repetition of the individual single trial N2-P2 peak-to-peak amplitudes of 70 FMS patients*

No relationship was observed between the N1 hand amplitude values and the habituation index in this sample of 70 FMS patients (r_s 0.224 p 0.062). (fig.27)

4.6.5 Relationship between the N2 peak and the Habituation Index

The relationship between the N2 peak-to-baseline amplitude and the habituation index was calculated. A strong relationship was observed as shown in figure 28 (N 85, r_s -0.435, $p < 0.001$).

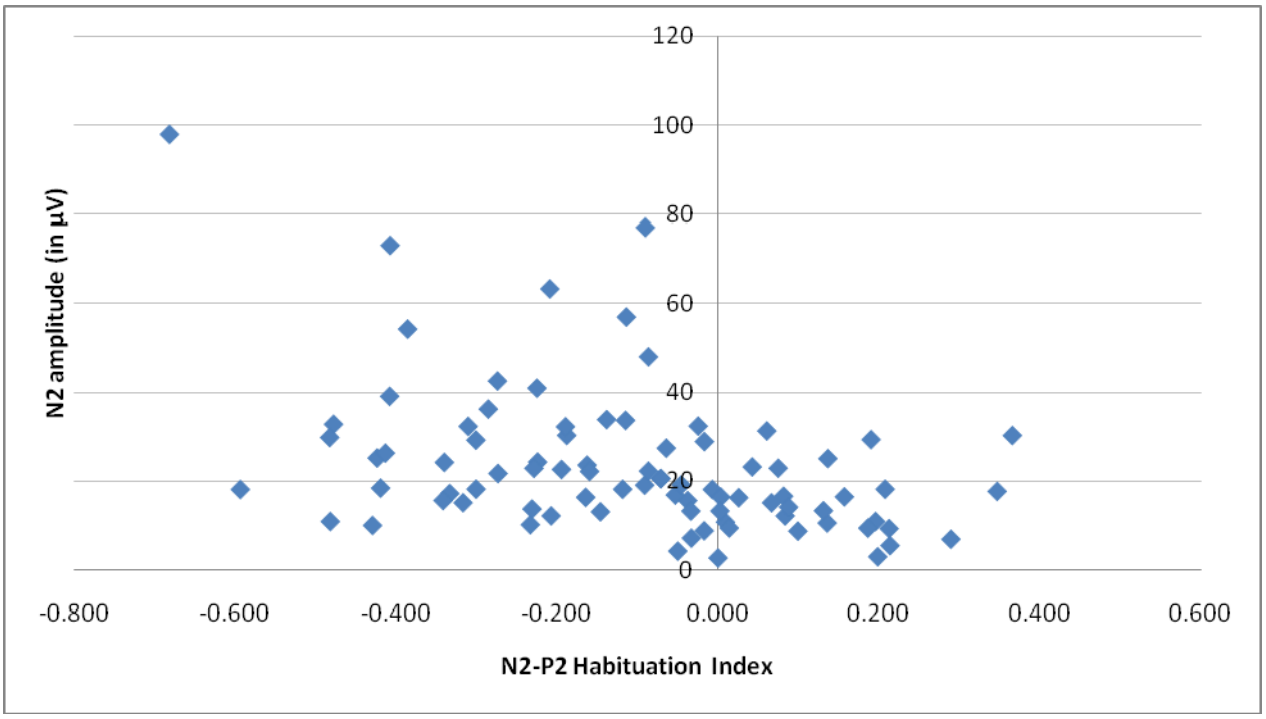


Fig. 28 *N2 peak-to-baseline amplitude values obtained after laser-stimulation on the hand in function of the calculated habituation index, based on first and third repetition of the individual single trial N2-P2 peak-to-peak amplitudes*

However, the N2 peak and the habituation index are not strictly speaking independent variables as the N2 amplitude is used for calculating the N2-P2 amplitude ratio. The average LEP N2 amplitude averaged over all recording blocks is evidently larger if the N2 amplitude in the first block is larger than in the last block. Some caution should be exerted in interpreting this results.

4.6.6 Habituation Index subgroups of fibromyalgia

FMS patients were classified on the basis of their habituation index (data not shown) as normal habituaters, reduced habituaters and non-habituaters (i.e. amplitude-amplificaters). Habituation subgroups with laser stimulation of the hand had the following sample-sizes: resp. 19-38-28; and with laser stimulation of the foot: resp. 10-42-33. Due to the small samples of each subgroup, we choose not to investigated them statistically, and would propose to further investigate clinical and electrophysiological differences between subgroups in the setting of a prospective study with larger patient population.

5 Discussion

5.1 Achieved results

Retrospective electrophysiological data of 85 FMS patients were recovered from a large patients' database collected over a 10-year period and newly submitted to an unbiased automated single trial analysis. The present study confirmed the usefulness of LEP as a diagnostic tool for nociceptive nervous system dysfunction in the context of FMS. LEP showed significantly increased amplitudes in FMS patients compared to healthy controls, whereas latencies remained similar. Not only N2-P2 (late components) amplitudes, but in particular N1 amplitudes (early component) were found to be higher in FMS than in healthy controls. This finding favours the hypothesis of a central sensitization phenomenon (large LEP amplitudes) explaining the general widespread pain symptoms in FMS, and invalidates the possibility of peripheral small fibre neuropathy (expected small LEP amplitudes) as a pathophysiological mechanism in this study's patient sample.

Furthermore, a direct relationship was found between age and LEP amplitudes explaining why in some FMS patients LEP amplitudes falsely may appear as small, and stressing the importance of a systematic correction for age when interpreting patients 'data.

From a technical point of view, the present data suggest that in FMS, LEP elicited from the hand may provide a more reliable and discriminative assessment than LEP elicited from the foot. Indeed, foot LEP are not significantly different between FMS patients and healthy controls and they are more difficult to recognize as their amplitude is almost two times smaller than hand LEP amplitude.

Finally, habituation to repetitive stimuli was found to be reduced in the FMS patient sample. The absence of a relationship between the habituation index and the N1 peak amplitude (arrival of nociceptive signal at the somatosensory cortex), suggests that abnormal habituation in FMS is a purely central phenomenon independent of peripheral nociceptive input.

5.1.1 Laser-evoked potentials as a diagnostic tool in fibromyalgia

5.1.1.1 Abnormal findings in fibromyalgia

The present study confirmed the diagnostic value of laser-evoked potentials for assessing the function of the nociceptive system. In most neurological conditions, an absence or decrease of LEP amplitudes is observed in relationship with a *lesion* of the nociceptive somatosensory pathways or brain areas, and the reliability of LEP for assessing these medical conditions has been repeatedly described (32). Retrospective data of the present study indicate that LEP may also be considered a valuable tool in medical conditions with a *dysfunction* of the nociceptive nervous system that includes an increase in the processing of nociceptive stimuli. From a clinical perspective, this means that LEP are not only appropriate to show loss of function (sensory deficit) but also gain of function (hypersensitivity). Few studies had shown this previously (42).

In more detail, the present study showed that *latencies of LEP components* are similar in FMS as in controls. This finding indicates that nerve conduction times of peripheral nociceptive afferents and central nociceptive pathways are normal in FMS. It also strengthens the data analysis of this study as similar peak latencies between FMS and controls ensure that the same peaks were compared between both groups. All other LEP studies also showed that latencies are normal in FMS. One can observe a small amplitude for multiples reasons but an increased latency, especially of N1, is mainly due (not to say pathognomic) of a peripheral small fiber neuropathy. We observed no differences in latencies of LEP components at the level of groups, which doesn't preclude that a few individual patients may have had prolonged latencies.

Then, the present data showed that *N2-P2 peak-to-peak amplitudes* were significantly larger in FMS patients compared to healthy controls (42.3 µV± 27 vs. 26.2 µV ± 14). An increased N2-P2 amplitude also was observed in FMS patients by other research groups as Gibson et al (1994), Lorenz et al (1996), Granot et al (2001), Garcia-Larrea et al (2002) and deTomasso et al (2011) (9, 10, 22, 26, 49). Increased N2-P2 LEP amplitude may be considered the signature of FMS. Of note, LEP in psychiatric patients with altered pain perception are found to be normal, thus differentiating psychiatric conditions from FMS (15).

Only one previous study (Lorenz et al 1996) described the *N1 LEP component* in FMS (49). All other studies in FMS failed to describe this component (9, 18, 36). The present study protocol enabled finding of the N1 peak through the use of supra-threshold laser stimuli, sufficient number of single trials, re-referencing of T3/T4 to Fz (temporal-frontal montage), trace superposition and the automated single trial approach. Statistical analysis of hand N1 peak-to-baseline amplitude showed a significant difference between FMS (9.6 µV± 7) and control subjects (7.8 µV± 4). The failure to observe N1 in other studies (9, 18, 36) may be due to recording conditions (temporalis muscle artefacts contaminating the temporal electrodes and a smaller SNR (15, 39, 51)) .

The *Habituation index* is a relatively new parameter to investigate habituation of the somatosensory system to repetitive stimuli. To our knowledge, only 3 previous studies described N2-P2 habituation in FMS (9, 17, 50). In healthy controls, the habituation index on the hand is approximately –0.30 corresponding to a 30% decrease in LEP magnitude between the first laser stimuli and the last laser stimuli. The habituation was found to be reduced in FMS at all stimulated sites in studies by Valeriani et al (2003), deTommaso et al (2011 and 2014) (9, 17, 50) and also in the present study, i.e. the decrease was less than expected or in some cases an increase of LEP amplitude over time was observed.
At present, normative values of habituation after laser stimulation on the foot in healthy subjects are unavailable. It may be concluded that the habituation index of hand (-0.11 ± 0.2) and foot (-0.06 ± 0.2) seem reduced versus available comparative data.

Granot et al (2001) described a supplementary waveform in LEP of FMS patients, occurring after the N2-P2 complex: *the P3 wave* (22). P3 peaks are elicited in particular recording circumstances unmet in the methodological set-up of the present experiment. They are due to factors external to the nociceptive pathways (attention, arousal, anxiety). Moreover, P3 peaks are difficult to be distinguished from P2 peaks, because they occur at similar latencies as the P2 positivity. Therefore, their identification was not the purpose of the present study.

The *Detection Rate* of laser stimuli delivered to the hand was 93% in the FMS group vs. 100% in the control group; and the detection rate of laser stimuli delivered to the foot was 83% in the FMS group vs. 94% in the control group. No other studies have described the detection rate of nociceptive stimuli in FMS before. Of interest, FMS patients seem to incompletely detect nociceptive somatosensory stimuli, though the electrophysiological correlate elicited by these stimuli is increased (N2-P2 amplitude). The significance of this finding remains unclear.

Mean *Reaction Times* of FMS patients were 561 ms ± 246 after hand stimulation and 825 ms ± 422 after foot stimulation. These values are considerably higher than those observed in the healthy control group (Hand: Right 414 ms ± 84 / Left 401 ms ± 87 -- Foot: 502 ms ± 346. Again, this is surprising given that the electrophysiological correlate of the reaction times, i.e. the latencies of the LEP components, is completely normal. It is not excluded that attentional bias may play a role. A, ANOVA comparing RT of FMS vs. HCs wasn't possible due to the lack of RT data in the HCs group.

5.1.1.2 Technical aspects of laser-evoked potential recording in fibromyalgia

Results of this retrospective research project indicated that *age as a covariate* may significantly influence the interpretation of LEP amplitudes. No significant correlations were observed between age and LEP latencies. When LEP amplitudes were corrected for age, the differences in LEP amplitude between FMS and HCs in this study were exacerbated. It is well established that LEP amplitude in healthy controls declines with age (15, 17, 31). To our knowledge, this negative correlation of LEP amplitudes with age has till now not been described in *FMS subjects.* Thus, small LEP amplitudes in a FMS patient should be interpreted with caution and first corrected for age before (falsely) assuming a lesion of the nociceptive system (such as small fibre neuropathy) (9, 22, 26, 49, 50). Based on current data, it can be concluded that age is an important covariate to take into account when interpreting LEP amplitudes in FMS patients, in clinical and research setting.

The *preferred site for laser stimulation* appears to be the *hand*, from the present research. Several arguments account for this finding. First, after laser stimulation of the foot, no significant differences were observed between the N2-P2 amplitudes of FMS patients (29.0 µV± 16) and controls (28.8 µV± 10). Second, the LEP components obtained after laser-stimulation of the hand were significantly correlated with those obtained after stimulation of the foot, thus reducing the interest of stimulating specifically the foot. Third, laser stimulation of the foot produces a N2-P2 peak of 70% of the amplitude of the N2-P2 peak obtained by stimulating the hand, making the signal-to-noise ratio of hand recordings more interesting. The reason for this phenomenon may be that nociceptive signals coming from distal body sites present more jitter due to heterogeneous and low nerve conduction velocities of peripheral nociceptive nerves. In the words of Truini et al (2005) : a long conduction distance goes accompanied by a high signal dispersion along the Aδ-afferents, followed by a desynchronized volley presented at central synapses (31). This higher signal dispersion together with a lower free nerve ending density distally, could explain the smaller amplitude responses after foot stimulation compared to equal stimulation at the hand side (9, 26, 51).

Nevertheless, LEP *recording of the foot* may be needed when other diseases than FMS need to be excluded (such as small fibre neuropathy). Uceyler et al (2013) lay emphasis on the major importance of the investigated body region in FMS patients, because large and small nerve fibre impairments typically display a distal-to-proximal spread (7). Therefore, impairment in small fibre function would be first and most intensively expected at distal parts of the body i.e. the feet (7). To interpret correctly, LEP latencies after foot stimulation, they need to be corrected with the body height of each patient (not available from retrospective data) (9, 26, 31, 51).

Regarding side-to-side differences of LEP components, no differences could be observed for hand or foot data. Either side may be investigated and it can be considered as safe to *merge both sides* (left and right) as one group, when both recordings belong to the same patient.

5.1.2 Dysfunction of the nociceptive system in fibromyalgia: central sensitization or abnormal peripheral input or both?

Till now, it is proposed that FMS patients can be classified in (1) patients presenting with central sensitization phenomenon and (2) patients with lesions of the peripheral nervous system similar to small fibre neuropathy. The primary endpoint of this study was to verify the existence of these patient subgroups on a large data sample. The secondary endpoint was to derive suggestions for the pathophysiological mechanisms underlying FMS, based on the LEP abnormalities observed in our large FMS cohort.

As explained in the previous section, the unbiased automated single trial analysis of LEP recordings in fibromyalgia patients collected retrospectively from a larger database, showed that FMS patients had increased N1 and N2-P2 peaks compared to controls. This finding corroborates the hypothesis that FMS patients presented with central sensitization. Surprisingly, after correction for age (which was justified given the influence of age as a covariate) none of the FMS patients could be diagnosed with small LEP amplitudes compared to controls. This indicates that, in the present FMS patient sample, no typical SFN lesions of the nociceptive somatosensory system could be diagnosed, as been suggested previously by other authors (7, 17). However, this absence of a subgroup of patients with small LEP amplitude, does not preclude the existence of SFN or other subgroups in the present FMS sample, as suggested by the distribution of LEP amplitudes. LEP amplitude values in the FMS group were not normally distributed in contrast with those of the healthy control group (see also Truini et al 2005). This finding indicates that the nociceptive nervous system dysfunction in FMS may not be regarded as a homogeneous entity, but probably corresponds to different subgroups of patients with more or less increased amplitudes compared to healthy controls. Several hypotheses may explain the absence of FMS patients with peripheral nervous system lesion in our sample: (1) patients with small fibre neuropathies were correctly diagnosed and excluded from referral to the Algology laboratory for LEP examination; (2) patients with small fibre neuropathies discovered at a later stage were excluded from this sample.

Why is increased LEP amplitude the electrophysiological correlate of central sensitization?
Based on the exclusive relationship between the N2-P2 complex and the Aδ-nociceptor activation, as well as the fact that this main LEP complex expresses the saliency of noxious stimuli, it can be stated that the N2-P2 peak in LEP forms the electrophysiological representation of the protective mechanism in which higher cognitive functions favour noxious stimuli relatively to other perceived stimuli (49, 63). This enables the individual to focus his limited cognitive resources towards what is acutely important: signals of imminent harm. Also, the LEP amplitude is directly depending on the number of healthy neurons in the LEP generating brain areas (9, 26, 47, 51). Therefore, noxious stimuli induce greater activation of specific cortical areas in FMS (15). These findings may contribute to the pathophysiology of chronic pain in FMS patient and the increased LEP amplitude may be a signature of an overactivation of these natural phenomena. The N2-P2 peak is largely unspecific for the sensory modality of the eliciting stimulus, and reflects mainly cortical neural activities (39). An elevated N2-P2 peak (certainly in combination with reduced habituation) strongly suggests an increased level of activation of the CNS pathways and excitability in the cortical areas (10). It also reflects the stronger sensory and attentional processing (perceived intensity), even facilitation of the cerebral potentials evoked by repetitive noxious input (9, 49). Given the large ISI (> 3 seconds) of the laser stimuli, the attentional impact probably does not account *per se* for increased amplitudes. The N1 wave is the sole pre-perceptual response in LEP recordings and directly related to the ascending nociceptive input. Also, it is insensitive to attentional modulation and resistant to cognitively induced manipulation of pain sensation. Therefore it is an excellent parameter to assess sub- and early cortical pain processing (39). Given the increase of this component in FMS patients, it probably reflects an increased sensory input at cortical level, indicating peripheral impairment, but it is not sufficiently altered to explain the increase in N2-P2, therefore arguing for a simultaneously existing central impairment (15). Reduced habituation or even an increase of LEP amplitude over time was found in our FMS data set. From a physiological point of view, habituation phenomenon allows a progressive, advancing reduction of neuronal activation of the sensory cortex, with the purpose of avoiding brain overstimulation. The reduced habituation of cortical responses to laser stimuli suggests therefore alterations in the pattern of cortical excitability in FMS patients, resulting in a loss of this protection mechanism. Also, it could explain why FMS patients have an increased pain sensation after repetitive stimulations compared to healthy persons (9, 15, 18, 26, 36, 39, 47, 51). DeTommaso et al (2011) concluded that QST findings showing reduced habituation contributed to the pathophysiology of FMS (9). Reduced habituation confirms an (partial) absence of sensory cortical recruitment to repetitive noxious stimulation, facilitating a generalized increase in pain perception (9, 50). This is probably a manifestation of the central pain processing dysfunction in FMS (14, 17). It has been observed in other CSS, such as migraine, and might explain the symptomatic overlap between these conditions. In these other conditions, both increased neuronal excitability and reduced inhibition have been proposed as possible causes of the observed reduced habituation, but the origin remains contentious (9, 17, 50). Garcia-Larrea (2002) advanced LEP in distinguishing between neuropathic - and chronic pain (26). DeTommaso et al (2011) connected this expression with findings of a reduced habituation and proposed the HI as a parameter to make the distinction (9). Our findings after hand - and foot noxious stimulation support the statement of deTommaso et al (2011) that the reduced habituation and its underlying mechanisms are not confined to the tender points, but seemed to be a generalized phenomenon (9).

The relationship between clinical symptoms of chronic pain/central sensitization and electrophysiological data (LEP) has been described by deTommaso et al (2011) and by Granot et al (2001) who showed respectively correlations between the N2-P2 amplitude decline and the duration of the illness, and between N2-P2 amplitude and the amount of pain experienced by the FMS patients (9, 22).

Then, what are the arguments in favour of peripheral nociceptor dysfunction regardless of the present findings against this hypothesis?
Reduced epidermal nerve density in FMS patients, has been recently objectified and correlated to decreased N2-P2 amplitude at the hand (7, 17). These findings of small fibre dysfunction in FMS seem conflicting with the largely documented increase in LEP amplitude observed in most patients. The coexistence of both phenomena can be explained two-fold: (1) they concern different subgroups of FMS patients; or (2) they characterize a disease with simultaneous involvement at both peripheral and central nervous system levels.
The significant decrease in distal total and regenerating small fibre density, as shown by skin biopsy, is explained by some as proof that FMS should be reclassified as being a SFN (also observed in patients with diabetes and HIV-associated SFN) (2). Even though this approach deserves recognition, some findings oppose this classification proposal. Repetitive and robust data indicate that LEP amplitude is increased in a majority of FMS patients (7). Furthermore, lesions of the somatosensory system causing neuropathic pain have been correlated with *reduced* LEP amplitudes (26, 67). SFN has been associated with prolonged N1 latency and reduced N2-P2 amplitudes. However, in FMS, latencies are consistently found to be normal or even shortened. These different arguments indicate that FMS is probably not a SFN, but this doesn't exclude it from being a small fibre neuropathology (7). What can be suspected, is that impaired small fibre function is frequent among FMS patients and probably plays an important or additive role in the FMS pathophysiology. Other explanations could be: a common occurring mis-diagnosis of patients with FMS, actually having unappreciated SFN, or the concomitant incidence of SFN and FMS. One of these propositions, once justified, could explain the phenotypical heterogeneity and clinical symptom complexity of this illness, and could elucidate further the nociceptive system dysfunction at both peripheral and central level (17).

It seems therefore reasonable to classify FMS as a disease of the nociceptive nervous system, at least with central nervous system dysfunction (central sensitization) at central level, and possible peripheral nervous system involvement.

5.2 Limitations of this study

This is a research protocol investigating electrophysiological data that were acquired retrospectively.

A number of clinical factors that may have influenced the LEP recording cannot be controlled or quantified: stability of the experimental set-up, compressive clothing, smoking -, alcohol -, caffeine consumption - or medication use prior to the investigation. The subject inclusion may be biased: as recruitment of patients mainly originated from secondary or tertiary care centres. Some specific clinical characteristics could not be retrieved retrospectively from the patients' files: CNS medications (2), concomitant neurological diseases (like distal sensory deficits) or CSS (like migraine) (50), body height (31) and potential underlying causes of FMS.

Though the sample size of the present FMS population was large enough to allow statistical analysis, subgroup analysis was impossible due to lack of power (small size of subgroup samples).

From a methodological point of view, it is regrettable that laser-evoked potentials are not widely available. Due to their high cost and need for technical expertise, only a few specialized laboratories have access to LEP technology. Furthermore, LEP do not provide information on the exact level of lesion along the nociceptive pathways (15, 16, 41). Though some caution should be exerted regarding the interpretation of the N1 peak, i.e. a low reproducibility (due to N2 overlap in time and space, and temporalis muscle artefacts) and a high inter-subject variability (due to its small SNR) (15), the present methodological set-up and post-processing analysis is robust to avoid these limitations. (9, 15, 18, 36, 39, 47, 51)

Nonetheless, despite the methodological and technical limitations of this research protocol, the homogeneity of findings in the FMS groups, clear-cut differences from the control groups and unbiased investigator participation, strengthen the confidence that the observations described in this study are valid and representative of larger cohorts of FMS patients.

5.3 Future development

A prospective study should include :

- Larger sample size allowing subgroups according to endophenotypes of FMS
- Detailed clinical characteristics of FMS patients (to allow for clustering for defining subgroups with electrophysiological data)
- Analysis of sensitivity/ specificity of the automated single trials analysis in FMS

5.4 Significance of the present findings

- Our results challenge the current concept of FMS as a "merely" psychological disorder. Objective nociceptive fibre system dysfunctioning is shown at electrophysiological level in FMS (7).
- Our data may help to improve the clinical usefulness of LEP as a diagnostic tool for FMS.
- This study contributes to the nosological status of FMS: rheumatologic vs. neurologic - and central vs. peripheral nervous system disease.
- Reduced habituation of cortical responses to laser stimuli in FMS suggests alterations in the pattern of cortical excitability. These findings provide further support for the use of medications with effects on the CNS in the management of FMS (9).

6 Conclusion

At present, a heavy discussion is shaking different research groups specialized in FMS (Oaklander, Wolfe, Uceyler, deTomasso) concerning the possible involvement of peripheral small fibre neuropathy in the pathophysiology of FMS. From our point of view, these study results suggest that FMS may be a medical condition that develops itself within a biobehavioural model of reactivity to multimodal stimuli as a result of altered central neuronal excitability, as in other chronic pain syndromes. Our results challenge the still widespread concept of FMS as a 'merely' psychological disorder, because objective dysfunction of the nociceptive nervous system is shown electrophysiologically.

The concurrence of peripheral and central factors, the dysfunction of nociceptive small sensory fibres and of cortical zones electively devoted to pain modulation may all account for the complex pathophysiological mechanisms underlying FMS. The complex pathogenesis of FMS correlates well with its phenotypical heterogeneity. Different subgroups of patients or the concomitant occurrence within a patient of different central and peripheral factors balance the homeostasis of the nervous system. Small fibre pathology may be a PNS contributor to the complex pathophysiology of pain in FMS. However, the results of this retrospective study did not allow concluding if FMS itself is accompanied by SFN or if some SFN patients may be misdiagnosed as having FMS due to the unusual presentation of their symptoms as non–length-dependent SFN. Future studies in a larger cohort of patients are needed to confirm our data and to shed light on the relationship between SFN and FMS in order to better understand the aetiology of SFN in these patients and to optimize their treatment.

A reduced habituation in the course of laser stimulation may express a central mechanism of altered pain modulation, which correlates with the clinical appearance of FMS. In our opinion, these modifications may contribute to the complexity of FMS, but are in need of further investigation.

The complexity of FMS' pathophysiology, encompasses dysfunctioning of the nervous system and related dysfunctioning of neuro-endocrine, hormonal and immune systems leading to heterogeneous clinical presentations with issues related to pain, fatigue, cognition and many other associated symptoms. It calls for a highly innovative, integrative and at times unorthodox approach on the part of both clinicians and researchers. Though some specific statistical analyses may provide solution for diagnostic testing in the absence of gold standard (68), the availability of a gold standard for diagnosing FMS - still awaited by the scientific community - would represent a significant improvement in the assessment and therapeutical management of FMS. FMS diagnosis management in a decade may prove to be very different from what we know today.

7 Conflicts of interest statement

The author declares no conflict of interest with respect to this work.

L.P. and S.H. designed the study protocol. D.V.A. collected and encoded the data. D.V.A. and L.P. worked on data analysis and statistical analysis. D.V.A., L.P. and S.H. wrote and revised the manuscript. L.V. revised the manuscript.

8 Publication

The abstract (Section 1) will be submitted for poster presentation at the 14th National Congress of the French Society for the Study and Treatment of Pain (SFETD), which will take place on 20-22 November 2014 at Toulouse - Congress Centre (France).

9 List of abbreviations

ACG Anterior Cingulated cortex

ACR American College of Rheumatology

AFT Autonomic Function Testing

AnCoVa Analysis of Co-Variance

AnOVa Analysis of Variance

ASTA Automated Single-Trial Analysis

AUC Area Under the Curve

CFS Chronic Fatique Syndrome

CNS Central Nervous System

CoSy Systemic and Cognitive neuroscience

CPS Chronic Pain Syndromes

CSS Central Sensitivity Syndromes

CWP Chronic Widespread Pain

CWT Continuous Wavelet Transform

Df Degree of freedom

DTh Laser Detection - / perceptive Threshold

EEG ElectroEncephaloGraphy

EOG Electro-Oculo-Gram

EP Evoked Potential

ERPS Event Related PotentialS

FMS FibroMyalgia Syndrome

HCs Healthy Controls

HI Habituation Index

IBS Irritable Bowel Syndrome

ICA Independent Component Analysis

ICC Intraclass Correlation Coefficient

ICs Independent Components

IoNS Institute of NeuroScience

ISI Inter-Stimulus Interval

LBP Lower Back Pain (idiopathic)

LEP Laser-Evoked Potential(s)

LW5 Lets-Wave 5

MCS Multiple Chemical Sensitivity

MLR Multiple Linear Regression

N = Number =

N.A. Not Applicable

NCS Nerve Conduction Studies

NLS Neuropathic-Like Symptoms

NT NeuroTransmitter

PNS Peripheral Nervous System

PSPD Persistent Somatoform Pain Disorder

PTh Laser Pain Threshold

QST Quantitative Sensory Testing

RLS Restless Leg Syndrome

RT Reaction Time

SD Standard Deviation

SEP Somtatosensory Evoked Potentials

SFETD French Society for the Study and Treatment of Pain

SFN Small Fibre (poly-)Neuropathy

SI Primary Somatosensory cortex

SII Secondary Somatosensory cortex

SNR Signal-To-Noise Ratio

SS Symptom severity

TMJD Temporo-Mandibular Joint Dysfunction

VMpo Posterior Ventro-Medial nucleus

VPI Ventro-Postero-Inferior nucleus

VPL Ventro-Postero-Lateral nucleus

VUB Vrije Universtiteit Brussel

UCL Université Catholique de Louvain

ULB Université Libre de Bruxelles

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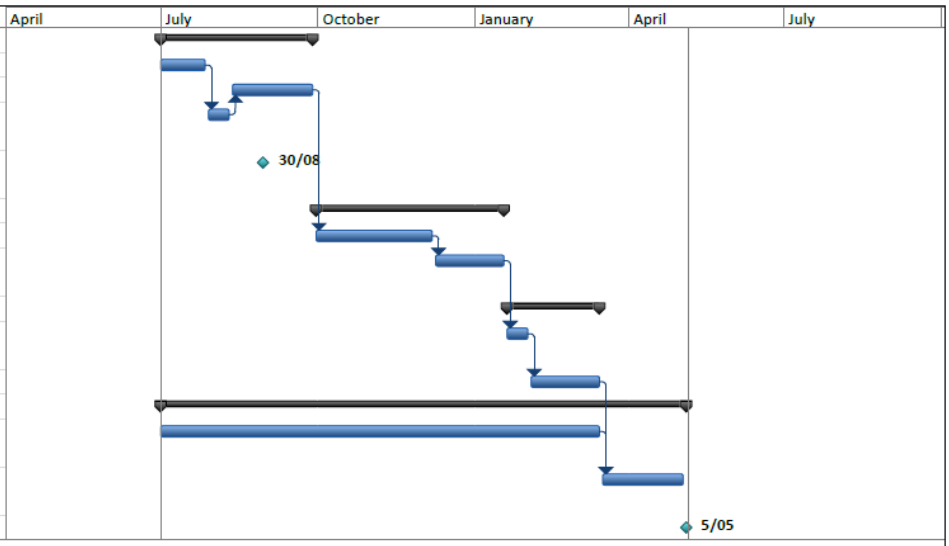
12 Appendices

12.1 Flowchart

ID	Task Name	Duration
1	**Literature study**	**65 days**
2	Literature study phase 1	20 days
3	Literature study phase 2	35 days
4	**Protocol preparation ethics commission**	10 days
5	*Protocol submission ethics commission*	0 days
6	**Database generation**	**80 days**
7	Database assembly	50 days
8	Contact physicians and patients	30 days
9	**Statistical analysis**	**40 days**
10	Prestudy of statistical methods and tools	10 days
11	Database analysis	30 days
12	**Documentation**	**220 days**
13	On-the-fly documentation	185 days
14	Thesis writing phase	35 days
15	*Thesis hand-in*	0 days



Project: study LEP in FM
Date: Wed 7/08/13

Task	External Milestone	Manual Summary Rollup
Split	Inactive Task	Manual Summary
Milestone	Inactive Milestone	Start-only
Summary	Inactive Summary	Finish-only
Project Summary	Manual Task	Deadline
External Tasks	Duration-only	Progress

Page 1

12.2 Protocol

Protocol to the ethics committee of CHU Brugmann

Laser-evoked potential characteristics in fibromyalgia

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1. Introduction

1.1. Fibromyalgia

Fibromyalgia (FM) is a common chronic disorder of the central nervous system (CNS) pain processing. This debilitating disorder affects the patient's ability to work and engage in everyday activities and imposes a large economic burden to society. Its cardinal symptoms are chronic widespread pain, fatigue, unrefreshed awakening and cognitive dysfunction. (1)

Unfortunately, FM remains undiagnosed in an estimated 75% of all affected individuals, leading to inadequate management of the condition. The current clinical approach in diagnosing FM is based on the 2010 American College of Rheumatology (ACR) classification criteria; this includes a widespread pain index and symptom severity scale. (1, 2)

Extensive research suggests a neurochemical imbalance in the CNS as origin, causing a global aberrant central pain processing. This and multiple other factors lead to an abnormal operation of both the ascending and descending nociceptive pathways, resulting in 'central amplification' of pain perception. (1, 3) Laser-evoked potentials (LEP) represent currently the gold standard for exploring the ventrolateral spinothalamic pathway and in particular for examining the conduction of Aδ small nerve fibres implicated in thermonociceptive processing. (4, 5)

1.2. Laser-evoked potentials

A CO2 laser is a radiant heat source in far infrared, which sends powerful stimuli, which activate selectively Aδ and C nociceptors. Reproducibility of the stimulus is guaranteed, as absorption of the skin to radiation is almost complete (>99%) and reflectance very low. Research has shown that LEP reflects the functional state of the nociceptive pathways, which are related to the neural processing of pain perception. Despite a close relationship, LEP and subjective perception can be dissociated. (5, 6)

1.3. LEP findings

- Healthy individuals
  Normally, we observe a late LEP, which is a negative-positive complex, called N2-P2, with a maximum located at the vertex (Cz-A). This complex is preceded by an early negative N1, present on the contralateral temporal electrode. Endogenous P3 component (Pz-A) can be observed in attended conditions.
  The N2-P2 component has a latency of 200-500ms, greater for stimulating the foot than the hand, due to longer peripheral stimulus travel distance. The N2-P2 amplitude is likely to be modulated by attention of the subject (if focused, the amplitude is higher), and it is known to decrease with age. (4, 5, 7)

- Fibromyalgia
  There are 2 studies dedicated to examining LEP in FM patients. They describe an increased amplitude of P2 and N1. However, in a recent study of Uçeyler et al. (Brain 2013), electrical stimuli with concentric needle electrodes, specific for Aδ fibre activation, were used and a decrease in amplitude of the N2/P2 complex was observed. Also, the researchers of the Laboratory of Algology (IoNS - UCL) observe frequently in FM patients a reduction in amplitude and an increased latency. (5, 8, 9)

1.4. Hypothesis of the study

The aim of the present study is to examine if FM patients have a dysfunctional thermo-nociceptive system based on LEP recordings, as suggested by previous studies. (5, 8, 9)

2. Methodology

2.1. Patients

In this retrospective study all LEP recordings, acquired from 01/01/2004 till 31/12/2012 (approximately 1000 patient files), of the Laboratory of Algology in the department 'Systemic and cognitive neuroscience' (COSY) of the Institute of NeuroScience (IoNS), of the 'Université catholique de Louvain' (UCL), will be examined

Based on the clinical history of each patient that underwent LEP recordings, it will be determined which patients presented with the clinical diagnosis FM at the time of LEP recording. Most of these patients were referred through physicians of Cliniques universitaires St-Luc and Cliniques universitaires Montgodinne and their patient files may need to be accessed electronically.

Patients will be excluded from the present study in case of incomplete LEP recordings or recordings of poor quality, known existence of another neurological disorder, or use of psychotropic drugs that could possibly interfere (benzodiazepines) with the LEP recording. It is estimated that 50 LEP patent recordings will remain suitable for analysis in the present study.

The LEP and clinical data of these remaining patients will be collected and the referring doctors will be questioned about the current diagnosis of these patients. If insufficient clinical data can be collected through this procedure, then patients will be telephonically contacted for further questioning, after oral informed consent.

The research protocol will be submitted for approval to the Ethics Committee of CHU Brugmann, where data will be analyzed, as well as secondarily to the Ethics Committee of 'Université catholique de Louvain', for approval of collecting data.

2.2. CO_2 laser acquisitions

All LEP recordings were performed by the same examiner and with the same technical conditions over the period of 8 years. During 1 hour, the patient was placed in a comfortable chair,. All equipment associated with the production of the laser stimulus was kept outside of the visual field of the patient. Twenty scalp electrodes were positioned according to the 10-20 international system referenced to the linked earlobes (A1-A2). (10) An electrooculogram (EOG) was recorded, to control for eye movement artefacts. The patient had to leave his eyes open and fixate a point, in order not to disturb the signal with potential large α-waves. Stimuli were applied to the dorsal side of the left hand and right foot. The infrared laser beam had a pulse duration of 10-50 ms and a beam diameter of 10 mm. The laser stimulus intensity was determined in a standardized manner as twice the perceptive threshold of each patient. The perceptive threshold was determined by gradually increasing and decreasing the intensity of each laser stimulus. The mean pain threshold of healthy subjects is 7 mJ/mm^2 and an intensity higher than 10 mJ/mm^2 is potentially hazardous. In normal skin, the sensation evoked by laser stimuli near pain threshold is comparable to a weak pinprick. In order to obtain reproducible evoked potentials, it was necessary to use supra-threshold stimuli, which are usually perceived as slightly burning. The irradiated target was slightly shifted from trial-to-trial, with an inter-stimulus interval randomly varying between 5-10 s. (4, 5, 11, 12)

2.3. Data analysis

In total EEG data were obtained from, 20-30 laser stimulations on each examination site. These raw data will be re-analyzed in the present study using the following method: baseline correction, band-pass filtering and artefact correction by using independent component analysis. The amplitude and latency of each LEP peak will be estimated through automated single-trial analysis as described in Hatem et al. (2012). This method allows for objective evaluation of evoked potential data. (13)

<u>Independent variables</u>

Normative data will be obtained from a control group, matching in sex and age, issued from the database of the Laboratory of Algology at UCL (Brussels, Belgium).

Dependent variables (7, 11)

- The baseline-to-peak amplitude of the main LEP positive and preceding negative component (P2 and N2 amplitude)
- The baseline-to-peak amplitude of N1 and P3 if present
- The peak-to-peak amplitude of the N2/P2 complex
- The peak latency of the P2, N2, N1 and P3 component
- The reaction-time (RT): measured as the time between the opening of the laser shutter and the pressing of the micro-switch (right hand) (12)
- The perceptive threshold for Aδ and C nociceptor activation

2.4. Statistical analysis

Cluster analysis based on the dependent variables will lead to the discrimination of different subgroups of FM patients. Correlation analysis between clinical and demographic factors and dependent variables will be carried out within subgroups to assess the relationship between electrophysiological abnormalities and clinical relevance. ANOVA between subgroups of FM patients will assess differences between dependent variables.

3. Hypothesis

We hypothesize that all FM patients do not have amplified LEP responses. Other LEP abnormalities could be linked to clinically distinct subgroups of FM.

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12.4 Informed consent

12.4.1 Information sheet for the patient

Madam, Miss, Sir,

You will participate in the study "Do all fibromyalgia patients have abnormal laser evoked potentials?". This research protocol was designed to determine abnormalities in laser-evoked potential recordings in fibromyalgia patients. The doctor responsible for this study is Prof. Dr. S. Hatem, head of clinic for physical medicine and rehabilitation / Pain Clinic - UHC Brugmann - Van Gehuchtenplein 4 - 1020 Brussel. This document is intended to provide you with all important information, to allow you to give your consent in participation of this study, with full knowledge of the facts.

You have been contacted because for clinical reasons you underwent Laser Evoked Potentials recordings by Prof. Dr. Plaghki, during the period 2004-2012, in the Laboratory of algology in the department 'Systemic and cognitive NeuroScience' of the Institute of NeuroScience of the 'UCL',- Cliniques universitaires St. Luc (Brussels).

The aim of the present research project is to identify significant abnormal electroencephalographic findings linked to fibromyalgia during the recording of laser-evoked potentials. The data previously recorded will be re-analyzed in order to use it as a diagnostic tool in fibromyalgia patients. Fibromyalgia is a common chronic pain disorder debilitating in everyday life and work. It deserves to be diagnosed in all affected individuals, so that patients can be adequately treated.

Your LEP recordings and clinical history will been studied, and the referring doctors may be questioned about your current diagnosis. However, it would be an enormous asset if we could collect more clinical data from you.

In order to achieve this, we will contacting you by phone for further questioning. This is a one-time intervention and we accept your possible refusal without questioning your motivation. However, we hope you realize the positive outcome of this research will almost entirely depend on the correct acquisition of these data.

The questions, that may be asked include: current diagnosis of your pain disorder, any other known neurological disorder, medication use, your clinical history and active pathologies. This survey will take 15 minutes maximum. Unfortunately, there is no monetary compensation for your time.

Your participation in this study has no effect on future medical aid and quality of care, that will be provided to you. The advice of the biomedical ethics committee of Cliniques universitaires St. Luc and CHU Brugmann has been requested for this research protocol and an information sheet was prepared.

The medical confidentiality and the legislative requirements of privacy will be respected (in accordance with the Belgian law of 8/12/1992), as well as the patients' rights (in accordance with the law of 22/08/2002) and the law of 07/05/2004 regarding experiments on humans.

You can decline now or at any time stop your participation for any reason whatsoever; and this without any disadvantage or liability, now or in the future.

Before taking part, we ask you verbally to provide your consent. You hereby declare that the purpose and duration of the inquiry is clear to you, as are the expected benefits and predictable limitations. Your data will remain strictly confidential at all times. Also you can ask at any time additional information to Prof Dr. Plaghki.

We thank you for your participation in this study,

Prof. Dr. Hatem	Prof. Dr. Plaghki	Dominique Van Assche
head of pain clinic (UHC Brugmann)	Prof Emer. Institute of Neuroscience (UCL)	final year medical student (VUB)
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Questions

1. Confirmation name, date of birth and date of LEP recording.

2. What is the current diagnosis of your pain disorder? How have your symptoms evolved since the LEP recording was made?

3. Before or after the moment of recording, were you diagnosed with any other neurological disorder? Have you ever experienced any of the following symptoms: paraesthesia, polydipsy/polyphagy/polyury, loss of sensation in (a part of) a limb, muscle weakness, loss of proprioception, muscle wasting, disturbed/diminished sensation, gait abnormalities ?

4. Can you remember if you were taking sleep medication or muscle relaxants at the time of LEP recording?
eg. alprazolam (xanax), bromazepam), clorazepaat (tranxene), cloxazolam (akton), diazepam (valium), loprazolam (dormonoct), lorazepam (temesta/serenase), lormetazepam (loramet/noctamid/stilaze), prazepam (lysanxa),tetrazepam (epsipam/myolastan)...

5. What is you clinical medical and surgical history (antecedents)? What are your active pathologies for the moment?

12.4.2 Feuille d'information au patient

Madame, Mademoiselle, Monsieur,

Vous participerez à l'étude« Est-ce que tous les patients atteints de fibromyalgie ont des potentiels évoqués laser anormale? ». Ce protocole de recherche a été conçu pour déterminer des anomalies dans des enregistrements potentiels évoqués laser chez des patients atteints de fibromyalgie. Le médecin responsable de cette étude est le Professeur Dr. S. Hatem, chef de clinique de médecine physique et réadaptation / Clinique de la douleur - CHU Brugmann - Van Gehuchtenplein 4-1020 Bruxelles. Ce document est destiné à vous fournir toutes les informations importantes, pour vous permettre de donner votre consentement à la participation de cette étude, en pleine connaissance des faits.

Vous avez été contacté parce que, pour des raisons cliniques, vous avez subis des enregistrements laser potentiels évoqués par le professeur Plaghki, au cours de la période 2004-2012, dans le laboratoire de l'algologie, dans le département « Systemic and cognitive NeuroScience » de l' « Institute of NeuroScience » de l'UCL, Cliniques universitaires St. Luc (Bruxelles).

L'objectif du projet de recherche actuel est d'identifier les résultats électroencéphalographiques anormaux significatifs liés à la fibromyalgie lors de l'enregistrement des potentiels évoqués laser. Les données précédemment enregistrées seront ré-analysés afin de l'utiliser comme un outil de diagnostic chez les patients atteints de fibromyalgie. La fibromyalgie est un trouble de la douleur chronique débilitante dans la vie quotidienne et du travail. Il mérite d'être diagnostiqué chez toutes les personnes affectées, de sorte que les patients peuvent être traités de manière adéquate.

Vos enregistrements PEL et l'histoire clinique seront été étudiés, et les médecins traitants peuvent être interrogés au sujet de votre diagnostic actuel. Cependant, il serait un atout énorme si nous pouvions recueillir plus de données cliniques de vous. Pour ce faire, nous allons vous contacter par téléphone pour un nouvel interrogatoire. Il s'agit d'une intervention ponctuelle et nous acceptons votre refus possible sans remettre en cause votre motivation. Cependant, nous espérons que vous vous rendez compte de l'issue positive de cette recherche sera presque entièrement dépendre de l'acquisition correcte de ces données.

Les questions, qui peuvent être posées comprennent: le diagnostic actuel de votre trouble de la douleur, tout autre trouble neurologique connu, l'utilisation des médicaments, votre histoire clinique

et les pathologies actives. Cette enquête prendra 15minutes maximum. Malheureusement, il n'y a pas décompensation monétaire pour votre temps.

Votre participation à cette étude n'a pas d'effet sur l'aide médicale future et la qualité des soins, qui seront mis à votre disposition. L'avis du comité d'éthique biomédicale des Cliniques universitaires Saint-Luc et le CHU Brugmann a été demandée pour ce protocole de recherche et une fiche d'information a été préparée.

Le secret médical et les exigences législatives de la vie privée seront respectés (conformément à la loi belge du 8/12/1992), ainsi que les droits des patients (conformément à la loi du22/08/2002) et la loi 07/05/2004 concernant des expériences sur des humains.

Vous pouvez refuser maintenant ou arrêter à tout moment votre participation pour aucun raison, et ceci sans aucun désavantage ni responsabilité, ni maintenant ni dans l'avenir.

Avant de participer, nous vous demandons de vous mettre d'accord verbalement. Vous déclarez que le but et la durée de l'enquête est claire pour vous, de même que les bénéfices attendus et les limites prévisibles. Vos données resteront strictement confidentielles à tout moment. Aussi, vous pouvez demander à tout moment des informations supplémentaires de la Prof Dr Plaghki.

Nous vous remercions pour votre participation à cette étude,

Prof. Dr. Hatem	Prof. Dr. Plaghki	Dominique Van Assche
chef de clinique de la douleur (UHC Brugmann)	Prof Emer. Institut des neurosciences (UCL)	étudiante en médecine de la dernière année (VUB)
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Les questions

1. La confirmation de nom, date de naissance et la date de l'enregistrement du PEL.
2. Quel est le diagnostic actuel de votre trouble de la douleur? Comment vos symptômes évolué depuis l'enregistrement du PEL a été fait?
3. Avant ou après le moment de l'enregistrement, avez-vous été diagnostiqué avec un autre trouble neurologique? Avez-vous déjà connu l'un des symptômes suivants: paresthésie, polydipsie/polyphagie/polyurie, perte de sensation dans(une partie de) un membre, une

faiblesse musculaire, la perte de la proprioception, fonte musculaire, perturbé sensation/diminution, anomalies de la démarche?

4. Pouvez-vous rappeler si vous preniez des médicaments pour dormir ou des relaxants musculaires au moment de l'enregistrement du PEL?
   ex.alprazolam (xanax), bromazepam), clorazepaat (tranxene), cloxazolam (akton), diazepam (valium), loprazolam (dormonoct), lorazepam (temesta/serenase), lormetazepam (loramet/noctamid/stilaze), prazepam (lysanxa),tetrazepam (epsipam/myolastan)...
5. Quelle est votre histoire médicale et chirurgicale cliniques(antécédents)? Quels sont vos pathologies actives pour le moment?

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All authors would like to thank every patient or healthy volunteer for his/her participation in past, current and future research.

Someone I certainly shall not forget to thank is Prof. Mouraux: for warmly welcoming me in the UCL and for his endless patience in sharing his extensive knowledge of biomedical software in times of 'crisis'. Also, I would like to thank the security guard of the laboratory of the IoNS, for saving me from lock-down when I had been working again too late.

I would like to express, once additionally, my gratitude towards Prof. Hatem and Prof. Plaghki.
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To Dr. E. Jansen goes my amicable gratitude, for her many ‘experience qualified contributions’.

My friends and family, I thank you for supporting me these last few years. I will never forget that.

Finally, last but surely not least: my affectionate gratitude goes to the love of my life Bart; without your unwavering optimism, sweet encouragements and practical tips and tricks, some days would have ended in even longer nights. I hope someday to reimburse you for all the dedication you have given me unconditionally these last 5 years.

Thank you all !

Dominique Van Assche

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