Title: Psychophysical and electrophysiological evidence for nociceptive dysfunction in CRPS

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2 Summary (25 words)

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4 Psychophysical and electrophysiological assessments of nociception using

5 laser stimulation reveal a marked dysfunction of thermo-nociceptive pathways

6 in the affected limb of patients with chronic CRPS.

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ABSTRACT

The aim of this study was to assess the function of the thermo-nociceptive system in 25 patients with long-lasting, medium to severe refractory CRPS-1 using behavioral (detection rates and reaction times) and electrophysiological (event-related brain potentials) responses to brief (50 ms) and intense (suprathreshold for A δ -nociceptors) CO₂ laser stimuli delivered to the affected and contralateral limbs, and by comparing these responses to the responses obtained in the left and right limbs of age- and sex-matched healthy controls.

As compared to healthy controls and as compared to the contralateral limb, the detection rate of pricking pain related to the activation of A δ -fibers was markedly reduced at the affected limb. Furthermore, reaction times were substantially prolonged (>100 ms in 84% of patients and >300 ms in 50% of patients). Finally, the N2 and P2 waves of laser evoked brain potentials were significantly reduced in amplitude, and their latencies were significantly increased. Taken together, our results show that in the majority of patients with chronic CRPS-1, thermo-nociceptive pathways are dysfunctional. A number of pathological mechanisms involving the peripheral nervous system and/or the central nervous system could explain our results. However, the primary or secondary nature of these observed changes remains an open question.

KEYWORDS

CRPS, laser-evoked potentials, psychophysics, A δ fibers, neuropathic pain

INTRODUCTION

The symptoms of Complex Regional Pain Syndrome (CRPS) cluster into four distinct categories: (1) abnormal pain, (2) vasomotor and temperature changes, (3) sudomotor changes and edema and (4) motor dysfunction and trophic changes [13]. Two subtypes of CRPS are defined according to the absence (CRPS-1) or presence (CRPS-2) of an unequivocal nerve lesion. Although the absence of nerve lesion questions whether CRPS-1 is a true neuropathic pain condition [19, 38, 48], there is abundant evidence suggesting that neuropathological mechanisms do contribute to CRPS-1, at the level of the peripheral nervous system (PNS) [1, 36, 37, 55] and, possibly, also at the level of the central nervous system (CNS) (for a review see [20]).

Drummond et al. [5] searched for anatomical and histochemical signs of sensory and sympathetic nerve alterations in skin samples of eight patients with mechanical hyperalgesia related to CRPS-1. They concluded that, as compared to skin samples obtained from the contralateral limb, there was no difference in distribution density or change in neurochemical content of sympathetic and cutaneous nociceptive fibers. In contrast, Oaklander et al. [36] reported that CRPS-1 was associated with post-traumatic focal and persistent minimal distal nerve injury (MDNI), in particular, distal degeneration of small-diameter axons subserving nociception and autonomic function. In 17/18 patients, axonal densities were on average reduced by 29% at the affected limb, as compared to ipsilateral and contralateral control sites. In addition, van der Laan et al. [55] examined the sural nerve from the amputated legs of eight severely affected CRPS-1 patients and found a mild to moderate loss of C-fibers in 4/8 nerves. Albrecht et al. [1] also reported detectable

neuropathological findings in the surgically amputated extremities of two patients diagnosed with CRPS-1. However, in a large survey of 298 patients with upper-limb CRPS-1, Gierthmühlen et al. [11] found that about 60% of patients had “normal” small fiber afferent function, leading her to conclude that “an isolated small fiber neuropathy is unlikely to be a major mechanism in CRPS 1”.

Given these conflicting reports, the objective of the present study was to assess the function of thermonociceptive pathways in 25 patients with long-lasting, medium or severe CRPS-1, by comparing the psychophysical (reaction-times, rate of detection) and electrophysiological (event-related brain potentials) responses to brief infrared laser stimuli delivered to the affected vs. contralateral limb. Indeed, laser-evoked brain potentials (LEPs) are currently recognized as the most reliable diagnostic tool to assess the function of the spinothalamic system in humans [12, 52, 54].

METHODS

Subjects

Twenty-five patients diagnosed with CRPS-1 were recruited from the multidisciplinary Pain Clinic (Cliniques universitaires Saint-Luc, Brussels, Belgium). Diagnosis was based on the Budapest criteria [14]. Study inclusion was conditioned by the availability of medical records including detailed clinical examination and medical history in order to compute a CRPS severity score [13]. Exclusion criteria were history of a major psychiatric disorder or the inability to understand the testing procedures.

Data of healthy volunteer participants were extracted from a large database of our laboratory in order to match as close as possible the age and gender ratio of the patient group. These participants had no clinical history, symptoms or signs of peripheral or central nervous system disorder, and were not taking any medication at the time of testing or in the month before testing. As the main objective of the present investigation was to study the responses to brief and supraliminal laser stimuli directed to the affected and asymptomatic side in unilateral CRPS-1, we examined the responses from left and right limbs obtained in healthy controls with a strictly similar protocol. Informed consent was obtained from all volunteers. The study was approved by the local Ethics Committee and conducted in accordance with the principles of the Helsinki declaration.

Stimulus

Cutaneous heat stimuli were delivered using a CO₂ laser (Université catholique de Louvain, Louvain-la-Neuve, Belgium). Stimulus duration was 50 ms and beam diameter at target was 10 mm. The energy density of the laser stimulus was adjusted individually on the asymptomatic limb or on the left hand in healthy controls to be clearly supraliminal for A δ -nociceptor activation (9.5 ± 2.3 mJ/mm²). We did not assess thresholds on the affected side, as we expected that these thresholds would be potentially biased by emotional and cognitive factors.

The stimuli elicited a clear pricking and burning sensation in the asymptomatic limb of patients and in both limbs of healthy participants. This sensation was always detected with reaction times <650 ms when stimulating the hand and

with reaction times <750 ms when stimulating the foot (i.e. reaction times compatible with the conduction velocity of myelinated A δ -fibers). The same energy density was used at both sites within each subject. The laser beam was slightly shifted between each trial to avoid skin overheating and minimize nociceptor sensitization or habituation.

Electroencephalogram (EEG) recording

At least 30 stimuli were delivered at each stimulation site, in blocks of 10 trials separated by a short resting period lasting approximately one minute. The inter-stimulus interval varied between 8 and 15 s. The EEG was recorded from 19 Ag-AgCl electrodes evenly placed on the scalp according to the International 10-20 system referenced to linked earlobes. In addition, the electro-oculogram was recorded from two surface electrodes, one placed below the right lower eyelid and the other placed lateral to the outer canthus of the right eye. Signals were amplified and digitized (gain: 1000; filter: 0.06 – 75 Hz, sampling rate: 167 cps) using a PL-EEG recorder (Walter Graphtek, Germany).

Reaction times and detection rates

For each trial, participants were asked to press a button as soon as any type of sensation was perceived at the stimulation site. Reaction times (RTs) were used to distinguish between detection mediated by C-fiber input and detection mediated by A δ -fiber input. Specifically, RTs <650 ms when stimulating the upper limbs and RTs <750 ms when stimulating the lower limbs were considered as related to the detection of A δ -fiber input, whereas RTs $\geq$ 650 or

≥750 ms were considered as related to the detection of C-fiber input (and, hence, to the absence of detection of A δ -fiber input). In previous studies, we have shown that, at least in healthy individuals, reaction times can be used to distinguish between detections triggered by A δ - and C-fiber input, because of the great difference between the nerve conduction velocities of A δ - and C-fibers [3]. RTs exceeding 2500 ms, were considered as undetected. For each subject and stimulation site, two detection rates were computed. First, the absolute detection rate, corresponding to the proportion of detected trials regardless of reaction time. Second, the rate of detections with RTs <650 ms (upper limb) and <750 ms (lower limb), corresponding to the proportion of trials detected with RTs compatible with A δ -fiber activation.

Analysis of EEG data

The EEG data were processed offline using BrainVision Analyzer (Brain Products GmbH, Germany) and Letswave 5 (<http://letswave.webnode.com>). The EEG was band-pass filtered (0.1 – 20 Hz) using a zero-lag Butterworth filter, segmented into epochs extending from -0.5 s to +2.5 s relative to stimulus onset (512 bins) and baseline-corrected (reference interval -0.5 to 0 s). Epochs contaminated by eye blinks were rejected by visual inspection. Average waveforms were then computed for each participant and stimulation site. Based on their peak latency and scalp topography as originally defined by Treede et al. [51], three distinct peaks (N1, N2, P2) were characterized in the LEP waveforms obtained following stimulation of the hands, whereas two distinct peaks (N2, P2) were characterized in the LEP waveforms obtained following stimulation of the foot. The N1 latency and amplitude was measured at the temporal electrode contralateral (Tc) to the stimulated hand, referenced

to electrode Fz, following the recommendations of Valentini et al. [53]. The N2 latencies and amplitudes were measured at the vertex electrode Cz referenced to linked earlobes A1A2.

Single trial analysis: relating LEP amplitude and RT latency

For each electrode and each time bin of the LEP waveform, the relationship between LEP amplitude and RT was assessed using linear mixed models (LMM) [26, 47] as follows: $y = a \cdot x + b \cdot u + e$. The dependent variable y represents single-trial LEP amplitudes. The independent variable x represents RTs with $a \cdot x$ as the fixed effect. The quantity $b \cdot u$ represents the random effect taking into account variability between patients and stimulation sites. The term e represents the residual error. This procedure yielded time-courses of T-value, representing the strength of the relationship between LEP signal amplitude and RTs. To address the problem of multiple comparisons, the significance level (P value) was corrected using a false discovery rate (FDR) procedure [10].

Statistical analysis

All data are expressed as means $\pm$ standard deviation (SD). The analyses were performed separately for the lower and upper limb groups. A Two-Way Mixed ANOVA, with stimulated “limb” (affected vs. asymptomatic limb for CRPS-1 patients; left vs. right limb for healthy participants) as the within-subject factor and “group” (CRPS-1 patients vs. healthy participants) as the between-subject factor was used for the analysis of psychophysical and LEP data. When appropriate, Greenhouse-Geisser correction of degrees of freedom and contrast analyses were used. The Bonferroni-Dunn test adjusted

for pairwise comparisons according to the number of paired comparisons to be performed. Significance level was set at $p < 0.05$. The statistical analyses were performed using Aabel 3 (Gigawiz Ltd. Co. 2010) and Matlab R2006b (The MathWorks, USA).

RESULTS

Study population

Characteristics of patients and healthy participants are reported in Table 1. Most patients were middle-aged but two female patients were adolescents with CRPS-1 in the lower limb. There was no significant difference in age and gender between the patient/control and lower/upper limb groups. As in most previous studies on CRPS (review in [33]), females were overrepresented (72%), and the lower limbs were most often involved (72%). The frequency distribution of the CRPS Severity Scores (CSS; [13]), showed two peaks; a first peak at medium scores (0.5-0.6; 36% of patients) and a second peak at high scores (0.8-1.0; 45% of patients).

Psychophysical data

Psychophysical data of healthy subjects and patients are summarized in Table 2. In the lower-limb group, comparison of the total detection rate showed a significant interaction between the factors 'group' and 'limb' (main effect of 'group': $F = 3.675$, $p = 0.064$; main effect of 'limb': $F = 13.387$, $p = 0.0009$; interaction: $F = 12.655$, $p = 0.0011$). Comparison of the detection rate with $RT < 750$ ms (A δ -fiber detections showed a similar interaction between the factors 'group' and 'limb' (main effect of 'group': $F = 10.413$, $p = 0.003$; main effect of 'limb': $F = 38.812$, $p < 0.0001$; interaction: $F = 21.011$, $p < 0.0001$).

Finally, comparison of RT also showed an interaction between the factors 'group' and 'limb' (main effect of 'group': $F = 8.368$, $p = 0.007$; main effect of 'limb': $F = 29.835$, $p < 0.0002$; interaction: $F = 14.714$, $p = 0.0005$). Post-hoc comparisons revealed that, in CRPS patients, the total detection rate was significantly decreased (-29%; $T = 3.691$, $p < 0.001$), the detection rate with $RT < 750$ ms was even further decreased (-52%; $T = 6.337$, $p < 0.001$), and the average RT were significantly increased (+69%; $T = 4.729$, $p < 0.001$).

In the upper-limb group, the total detection rate in CRPS patients differed significantly from the total detection rate in healthy controls (main effect of 'group': $F = 7.452$, $p = 0.018$). There was no main effect of stimulated 'limb' ($F = 7.646$, $p = 0.171$) and no interaction between the two factors ($F = 8.129$, $p = 0.334$), indicating that the total detection rates were reduced in the patient group, regardless of the stimulated limb. The detection rate with $RT < 650$ ms showed a main effect of 'group' ($F = 14.899$, $p = 0.002$), a main effect of the factor 'limb' ($F = 46.667$, $p < 0.0001$) and an interaction between the two factors ($F = 46.667$, $p < 0.0001$). Similarly, RT showed a main effect of 'group' ($F = 14.173$, $p = 0.003$), a main effect of 'limb' ($F = 27.537$, $p = 0.0002$) and an interaction between the two factors ($F = 29.976$, $p = 0.0001$). Post-hoc comparisons revealed that, in CRPS-1 patients, the detection rate with $RT < 650$ ms was significantly decreased (-31%; $T = 2.811$, $p = 0.014$) and that the RT were significantly increased (+78%; $T = 6.831$, $p < 0.001$) when stimulating the affected vs. the unaffected contralateral limb.

Finally, in CRPS patients, the temperature of the skin at the affected site was, on average, cooler than the temperature of the skin at the contralateral site by 2.0 ± 1.1 °C (range +0.2°C to -4.8°C; $n = 23$).

Laser evoked brain potentials

Figure 1 shows the average LEP waveforms obtained from a typical CRPS-1 patient. The patient is a female aged 30 years with a severe CRPS-1 (CSS: 0.82) of the right foot following a Hemi-Castaining ligamentoplasty for the treatment of chronic lateral instability of the right ankle, 2.8 years before the evaluation. Laser stimuli directed to the left asymptomatic foot dorsum were always detected, and detected with a RT<750 ms in 84% of trials. The average RT was 497 ms. In contrast, laser stimuli directed to the right symptomatic foot dorsum, only 83% of laser stimuli were detected and only 1 of these stimuli was perceived with a RT<750 ms. The mean RT was 1191 ms, i.e. more than twice the latency following stimulation of the unaffected foot. Comparison of the LEP waveforms obtained following stimulation of the affected and unaffected foot shows that the latency of the N2 and P2 peaks is markedly increased following stimulation of the affected limb ($\Delta N2 = 30$ ms; $\Delta P2 = 42$ ms) and that the amplitude of the N2-P2 is markedly reduced ($\Delta N2-P2 = -14$ μV ; -50%).

In the lower-limb groups, a N2-P2 complex with latencies compatible with the conduction velocity of A δ -fibers was identified visually in all waveforms recorded in the healthy controls. In lower-limb CRPS-1 patients, the N2-P2 complex was identified in all waveforms obtained from the unaffected limb and in 16/18 waveforms obtained from the affected limb (Figure 2 upper panels). Comparison of N2 latencies showed a significant interaction between the factors 'group' and 'limb' (main effect of 'group': $F = 1.675$, $p = 0.0206$; main effect of 'limb': $F = 3.350$, $p = 0.077$; interaction: $F = 9.787$, $p = 0.004$).

Comparison of P2 latencies (main effect of 'group': $F = 0.147$, $p > 0.5$; main effect of 'limb': $F = 5.044$, $p = 0.032$; interaction: $F = 5.755$, $p = 0.023$) and comparison of N2-P2 amplitudes (main effect of 'group': $F = 0.381$, $p > 0.5$; main effect of 'limb': $F = 3.845$, $p = 0.059$; interaction: $F = 6.147$, $p = 0.019$) revealed the same interaction. Post-hoc comparisons showed that, in CRPS-1 patients, N2 latencies (+11%; $T = 2.912$, $p = 0.006$) and P2 latencies (+8%; $T = 5.755$, $p = 0.023$) were significantly increased when stimulating the affected limb; whereas the N2-P2 amplitude was significantly decreased (-30%; $T = 2.941$, $p = 0.006$).

In the upper-limb groups, an N2-P2 complex was identified visually in all waveforms recorded in the healthy controls and all waveforms recorded in patients (Figure 2 lower panels). The N2-P2 complex was preceded by an earlier N1 wave, maximal over central-parietal leads of the hemisphere contralateral to the stimulated side [53]. Of note, this lateralized N1 wave was not visible when stimulating the foot dorsum. The two-way mixed model ANOVA revealed no main effect of 'group' (N1 latency: $F = 0.336$, $p = >0.5$; N1 amplitude: $F = 2.806$, $p = 0.120$), no main effect of 'limb' (N1 latency: $F = 1.083$, $p = 0.318$; N1 amplitude: $F = 4.168$, $p = 0.064$) and no interaction between the two factors (N1 latency: $F = 1.326$, $p = 0.272$; N1 amplitude: $F = 1.382$, $p = 0.263$). Similarly, the two-way mixed model ANOVA revealed that the latency of the N2 wave did not significantly differ across conditions (main effect of 'group': $F = 1.340$, $p = 0.270$; main effect of 'limb': $F = 1.720$, $p = 0.214$; interaction: $F = 0.183$, $p > 0.5$) and that the latency of the P2 wave differed only marginally (main effect of 'group': $F = 1.088$, $p = 0.047$; main effect of 'limb': $F =$

4.935, $p = 0.046$; interaction: $F = 2.907$, $p = 0.114$). In contrast, comparison of N2-P2 amplitudes revealed a significant interaction (main effect of 'group': $F = 0.088$, $p > 0.5$; main effect of 'limb': $F = 3.829$, $p = 0.074$; interaction: $F = 6.443$, $p = 0.026$). Post-hoc comparisons showed that, in CRPS patients, the latency of the P2 wave was significantly increased (+10%; $T = 2.669$, $p = 0.018$) and the N2-P2 amplitude was significantly decreased (-34%; $T = 2.850$, $p = 0.013$) following stimulation of the affected limb.

Single-trial analysis of the relationship between LEP amplitude and RT latency

Conventional analysis of the latencies and amplitudes of LEP peaks obtained in the average waveforms showed that in the affected limb of CRPS-1 patients, the latency of the N2 and P2 waves was significantly increased, and the amplitude of the N2-P2 complex was significantly decreased. Parallel to these changes, the RT to stimuli delivered to the affected limb was significantly increased. For this reason, we conducted a supplementary analysis aimed at specifying the relationship between the amplitude of LEP waveforms and RT at the level of single-trials [16, 18]. The analysis was performed on the 21 patient EEG recordings for which we possessed complete original data files.

The results of this analysis are reported in Figure 3. A significant negative relationship between LEP amplitude and RT was observed maximally at Pz between 365 ms and 533 ms after stimulus onset, corresponding to the latency of the P2 wave. This relationship indicates that trials in which the stimulus elicited P2 waves of smaller amplitude were also trials in which the stimuli were detected with later RT.

Ultra-Late C-fiber related LEPs

In two patients with lower limb CRPS-1, laser stimuli delivered to the affected limb did not elicit a clearly identifiable LEP at latencies compatible with the conduction velocities of A δ -fibers. Interestingly, in these two patients, the stimulus elicited a later negative-positive complex at latencies compatible with the conduction of C-fibers (Figure 4).

The first patient was a 57 year-old female presenting with severe CRPS-1 (CSS: 0.88) at the right lower limb for 12.7 years, following a Maquet osteotomy of the right knee. Stimulation of the unaffected left foot dorsum clearly elicited LEPs compatible with the conduction velocity of A δ -fibers (N2 latency: 263 ms; P2 latency: 449 ms). However, this response was followed by a later negative-positive complex whose latency (N2': 1006 ms; P2': 1365 ms) was compatible with the expected latency of C-fiber LEPs. Furthermore, stimulation of the affected right foot dorsum did not elicit an identifiable LEP compatible with the conduction velocity of A δ -fibers, but did elicit a LEP compatible with the conduction velocity of C-fibers (N2': 1156 ms; P2': 1341 ms). Such as the scalp topography of A δ -fiber related LEPs, the C-fiber related N2' and P2' waves were symmetrically distributed over the two hemispheres and maximal at the scalp vertex. As shown in the lower part of Figure 4, the frequency distributions of RTs were bimodal when stimulating the unaffected limb (with a first peak of RTs around 400 ms compatible with the detection of A δ -fiber input and a second peak of RTs around 1200 ms compatible with the detection of C-fiber input). In contrast, almost all RTs obtained when stimulating the affected limb were compatible with the conduction of C-fiber

input. However, one should be cautious when interpreting these results, as RTs are not only dependent on the time required to conduct and process the sensory input, but also on the time required for sensorimotor integration and the production of motor output. Therefore, one cannot exclude that the observed increase in RTs observed in these patients was due to an abnormal increase in the time required for sensorimotor integration and motor output. However, it seems unlikely that this could lead to an increase in the latency of the elicited responses of several hundreds of milliseconds.

The second patient was a 62-year old male presenting with severe CRPS-1 (CSS: 0.88) after an ankle sprain about 6.1 years before evaluation. In this patient, an A δ -fiber LEP was identified when stimulating the unaffected limb (N2 latency: 258 ms; P2 latency: 508 ms; N2-P2 amplitude: 23.8 μ V) but not when stimulating the affected limb. Instead, a C-fiber LEP was clearly visible (N2' latency: 1060 ms; P2' latency: 1458 ms; N2'-P2' amplitude: 23.8 μ V). The total detection rate was 82% for both limbs. However, the detection rate with RTs <750 ms was 70% when stimulating the unaffected limb, and only 2% when stimulating the affected limb.

DISCUSSION

In contrast to the healthy volunteers, we found that in patients with CRPS-1, (1) the total detection rate of laser stimuli and (2) the rate of detections with reaction times compatible with the conduction velocity of A δ -fibers were both significantly reduced at the pathological limb as compared to the asymptomatic limb. Likewise, we found that reaction times were substantially prolonged in

patients versus controls (increased by more than 300 ms in 50% of patients). Analysis of the LEP waveforms showed that the latency of the N2 and P2 waves obtained from the pathological limb were significantly increased. Furthermore, the amplitude of the N2-P2 complex was significantly decreased in patients versus controls. Taken together, these results indicate a dysfunction of thermo-nociceptive pathways in CRPS-1. In the following paragraphs, we discuss whether this dysfunction results from changes at the level of the PNS, the CNS, or both.

Biophysical properties of the skin

The chronic inflammatory state in the upper skin layers held responsible for edema and trophic changes could have altered the thermophysical properties of the skin in such a way that absorption and transmission of the CO₂ laser radiation are reduced at the affected limb. Although we used stimuli that were clearly supra-threshold for AMH-2 nociceptors in the hairy skin of healthy individuals (i.e. the A δ -fiber related LEPs), changes in biophysical properties of the affected skin cannot be ruled out entirely.

An additional factor that could have contributed to the increase in RT and LEP latencies at the affected limb was the average reduction in skin temperature of $2.0 \pm 1.1^\circ\text{C}$ at the affected vs. contralateral limb. However, it seems unlikely as the latency and amplitude of the early N1 wave of LEPs elicited by stimulation of the upper limbs was not significantly different between affected and unaffected sides in CRPS-1 patients.

Peripheral neuropathy

1 Given the well-characterized clinical signs and laboratory tests demonstrating
2 autonomic and vasomotor dysfunction in the skin of the affected limb of CRPS-
3 1 patients, one could expect to find an associated dysfunction of peripheral
4 nociceptive fibers (for a review see [37]). However, the multiple interactions
5 between autonomic and nociceptive systems make it very difficult to define a
6 causal direction and disentangle primary and secondary mechanisms.

7 Our finding that, following stimulation of the affected limb, the latency of the N2
8 and P2 waves was significantly increased, and the amplitude of the N2-P2
9 complex was significantly reduced is compatible with an increased trial-to-trial
10 jitter of response latencies [42]. However, it seems unlikely that this
11 mechanism could have played a significant role as the latency and amplitude
12 of the earlier N1 wave was not significantly different between groups and
13 between sides in CRPS-1 patients. Furthermore, the standard deviations for
14 N2-P2 amplitudes and latencies were not different between the patient and
15 control groups and between the affected and unaffected sides in CRPS-1
16 patients (Table 2).

17 In many neuralgic conditions, intraepidermal nerve fiber (IENF) density is
18 reduced [17, 27]. This might conceivably be the most prominent feature that
19 explains all the phenomena we observed in CRPS-1. Indeed, a reduction in
20 IENF density may explain the drop in total detection rate, the reduction in the
21 A δ -nociceptor related detection rate [35, 41] and the reduction in N2-P2
22 amplitude. However, Drummond et al. [5], in a qualitative study of skin samples
23 taken from nine patients with CRPS-1, did not find any difference in the
24 distribution of several neuron-specific markers as compared with the skin
25 samples taken from the contralateral side or from healthy controls. Van der

Laan et al. [55] examined nerve biopsies from eight amputated CRPS-1 limbs and found only a slight decrease of myelinated fiber density and pathology of unmyelinated fibers in four sural nerves, leading them to conclude that there was “no consistent pathology of the peripheral nerves”. In contrast, Albrecht et al. [1] found evidence of widespread cutaneous neuropathologic changes, including a marked decrease in epidermal innervation in skin samples from the amputated upper and lower extremity of two patients with CRPS-1. To our knowledge, there are only two studies reporting on IENF density in CRPS-1 using skin punch biopsies stained with the pan-axonal marker PGP 9.5, a validated technic for diagnosing small fiber neuropathies [6]. The first study [36] included 18 CRPS-1 patients and reported a 30% median reduction of IENF density as compared to the contralateral limb. As pointed out by Jänig and Baron [20], in patients with diabetic and other peripheral neuropathies, a 30% reduction of IENF density does not normally lead to clinically detectable changes of thermal and nociceptive sensations. The second study [23] showed that the IENF density in the affected limb of 43 CRPS-1 patients was not significantly different from the IENF density at the thigh or at the calf, or the IENF density assessed in healthy controls. Furthermore, there was no significant difference in the mean IENF density between patients with normal and abnormal sensory thresholds for warm, cold, heat-pain and cold-pain. Thus, there is no clear evidence for a structural reduction in IENF density and, hence, for a structural alteration of the PNS.

To account for our results, two possibilities should be considered at the peripheral level. First, CRPS-1 could be related to changes in the function of peripheral nociceptors without any visible change in their structure. The

1 persistent inflammatory state of the skin could modify the normal functioning of
2 IENF [25]. Furthermore, CRPS-induced vasoconstriction may induce hypoxia,
3 lactate increase and acidosis, which could also contribute to nociceptor
4 dysfunction [2, 24, 45]. Second, CRPS-1 could be related primarily to changes
5 in the function of A δ -nociceptors, with a relative preservation of C-fiber
6 nociceptors. Indeed, the present study showed a marked reduction of detection
7 rates compatible with the conduction velocity of A δ -fibers, and a marked
8 alteration of the LEPs related to the activation of A δ -fibers. Furthermore, in
9 some patients, the disappearance of the A δ -fiber related LEP led to the
10 appearance of a later C-fiber related LEP, suggesting that the dysfunction
11 affected A δ -fibers but not C-fibers (see [58] for a case reporting a similar
12 finding). Importantly, because the number of C-fibers is thought to greatly
13 exceed the number of A δ -fibers [39, 46], a selective structural alteration of A δ -
14 fiber IENFs could go largely unnoticed using currently available histological
15 techniques to analyze skin biopsies which only assess the total IENF density,
16 as these are unable to distinguish between C-fiber and A δ -fiber free nerve
17 endings. This interpretation is also compatible with the results of Gierthmühlen
18 et al. [11] showing no impairment of thermal thresholds in approximately 60%
19 of 298 patients with upper-limb CRPS-1. Indeed, because these thresholds
20 were assessed using a large (900 mm²) Peltier thermode and slow heating
21 ramps, these thresholds were likely to mainly reflect the function of C-fibers
22 [22, 59].

24 Central nervous system dysfunction

25 There is accumulating evidence that the CNS plays an important role in the

1 maintenance and, possibly, in the initiation of the symptoms associated with
2 CRPS-1 [33]. At the spinal level, Del Valle et al. [4] found microglial and
3 astrocytic activation as well as significant posterior horn cell loss in autopsy
4 tissue from the cervical, thoracic and lumbar spinal cord of a patient with
5 longstanding CRPS-1. Using magnetoencephalography, Walton et al. [57]
6 reported changes in the power spectrum of the recorded signals interpreted as
7 reflecting a neurological “disconnection syndrome” resulting from a
8 disturbance in thalamocortical interplay. An involvement of the CNS is also
9 suggested by a number of neuropsychological symptoms associated with
10 CRPS-1 like glove stocking sensory loss, trend to asomatognosia, referred
11 sensations, impaired representation and discriminative abilities of the
12 pathological limb and intentional motor dysfunction [30, 34, 43, 44].

13
14 Impaired perception and utilization of the affected limb in CRPS is consistent
15 with studies showing abnormal reorganization of the cortical activity in areas
16 involved in sensory-motor functions [21, 31, 32, 40, 56]. Importantly, such
17 changes could have contributed to the results of the present study, as these
18 would predict an increased latency and decreased amplitude of the responses
19 to nociceptive stimuli. For instance, the observed reduction in the somatotopic
20 map of the affected limb could of course result from reduced afferent input (as
21 observed in amputees with phantom limb pain [7]) or pain-induced reduction in
22 responsiveness of neurons in somatosensory pathways [11]. Importantly, such
23 a reduction in neuron responsiveness could be related to the fact that CRPS
24 patients tend to ignore the affected limb and attentional effort is needed to
25 move it [8]. Several studies have shown that, in healthy subjects, LEPs elicited

1 by stimulation of the attended limb are enhanced as compared to LEPs elicited
2 by stimulation of the unattended limb [9, 28, 29], as observed in our CRPS
3 patients. However, such an interpretation would not account for the
4 dissociation between a marked alteration of the responses to A δ -fiber input
5 and a relative preservation of the responses to C-fiber input, possibly even
6 leading to an “unmasking” of C-fiber LEPs in some patients.

7
8 In summary, the present results show that in most patients with chronic CRPS-
9 1, the thermo-nociceptive system is markedly dysfunctional. Whether the
10 observed dysfunction results from pathological mechanisms involving the PNS,
11 the CNS or both remains an open question.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest

REFERENCES

1. Albrecht PJ, Hines S, Eisenberg E, Pud D, Finlay DR, Connolly MK, Paré M, Davar G, Rice FL. Pathologic alterations of cutaneous innervation and vasculature in affected limbs from patients with complex regional pain syndrome. *Pain* 2006;120:244–66.
2. Birklein F, Weber M, Ernst M, Riedl B, Neundörfer B, Handwerker HO. Experimental tissue acidosis leads to increased pain in complex regional pain syndrome (CRPS). *Pain* 2000;87:227–34.
3. Churyukanov M, Plaghki L, Legrain V, Mouraux, A. Thermal Detection Thresholds of Aδ- and C-Fibre Afferents Activated by Brief CO₂ Laser Pulses Applied onto the Human Hairy Skin. *PLoS ONE* 2012;7: e35817.
4. Del Valle L, Schwartzman RJ, Alexander G. Spinal cord histopathological alterations in a patient with longstanding complex regional pain syndrome. *Brain Behav Immun* 2009;23:85–91.
5. Drummond PD, Finch PM, Gibbins I. Innervation of hyperalgesic skin in patients with complex regional pain syndrome. *Clin J Pain* 1996;12:222–31.
6. England JD, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, Cohen JA, Asbury AK, Szigeti K, Lupski JR, Latov N, Lewis RA, Low PA, Fisher MA, Herrmann DN, Howard JF, Lauria G, Miller RG, Polydefkis M, Sumner AJ. Practice Parameter: evaluation of distal symmetric polyneuropathy: role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. *Neurology* 2009;72:177–84.
7. Flor H, Nikolajsen L, Staehelin JT. Phantom limb pain: a case of maladaptive CNS plasticity? *Nat Rev Neurosci* 2006;7:873–81.
8. Frettlöh J, Huppe M, Maier C. Severity and specificity of neglect-like symptoms in patients with complex regional pain syndrome (CRPS) compared to chronic limb pain of other origins. *Pain* 2006;124:184–89.
9. Garcia-Larrea L, Peyron R, Laurent B, Mauguière F. Association and dissociation between laser-evoked potentials and pain perception. *Neuroreport* 1997;8:3785–89.
10. Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 2002;15:870–78.

- 1 11. Gierthmühlen J, Maier C, Baron R, Tölle T, Treede RD, Birbaumer N,
2 Hüge V, Koroschetz J, Krumova EK, Lauchart M, Maihöfner C, Richter H,
3 Westermann A. The German Research Network on Neuropathic Pain
4 (DFNS) study group. Sensory signs in complex regional pain syndrome
5 and peripheral nerve injury. *Pain* 2012;153:765–74.
6
- 7 12. Haanpää M, Attal N, Backonja M, Baron R, Bennett M, Bouhassira D,
8 Cruccu G, Hansson P, Haythornthwaite JA, Iannetti GD, Jensen TS,
9 Kauppila T, Nurmikko TJ, Rice AS, Rowbotham M, Serra J, Sommer C,
10 Smith BH, Treede RD. NeuPSIG guidelines on neuropathic pain
11 assessment. *Pain* 2011;152:14–27.
12
- 13 13. Harden RN, Bruehl S, Perez RS, Birklein F, Marinus J, Maihöfner C,
14 Lubenow T, Buvanendran A, Mackey S, Graciosa J, Mogilevski M,
15 Ramsden C, Chont M, Vattine JJ. Development of a severity score for
16 CRPS. *Pain* 2010;151:870–76.
17
- 18 14. Harden RN, Bruehl S, Perez RS, Birklein F, Marinus J, Maihofner C,
19 Lubenow T, Buvanendran A, Mackey S, Graciosa J, Mogilevski M,
20 Ramsden C, Chont M, Vattine JJ. Validation of proposed diagnostic criteria
21 (the “Budapest Criteria”) for Complex Regional Pain Syndrome. *Pain*
22 2010;150:268–74.
23
- 24 15. Hatem SM, Attal N, Ducreux D, Gautron M, Parker F, Plaghki L,
25 Bouhassira D. Clinical, functional and structural determinants of central
26 pain in syringomyelia. *Brain* 2010;133:3409–22.
27
- 28 16. Hatem SM, Hu L, Ragé M, Gierasimowicz A, Plaghki L, Bouhassira D,
29 Attal N, Iannetti GD, Mouraux A. Automated single-trial assessment of
30 laser-evoked potentials as an objective functional diagnostic tool for the
31 nociceptive system. *Clin Neurophysiol* 2012;23:2437–45.
32
- 33 17. Herrmann DN, Griffin JW, Hauer P, Cornblath DR, McArthur JC. Epidermal
34 nerve fiber density and sural nerve morphometry in peripheral
35 neuropathies. *Neurology* 1999;53:1634–40.
36
- 37 18. Hu L, Mouraux A, Hu Y, Iannetti GD. A novel approach for enhancing the
38 signal-to-noise ratio and detecting automatically event-related potentials
39 (ERPs) in single trials. *Neuroimage* 2010;50:99–111.
40
- 41 19. Jänig W, Baron R. Complex regional pain syndrome: mystery explained?
42 *Lancet Neurol* 2003;2:687–97.
43
- 44 20. Jänig W, Baron R. Is CRPS I a neuropathic pain syndrome? *Pain*
45 2006;120:227–29.
46
- 47 21. Juottonen K, Gockel M, Silen T, Hurri H, Hari R, Forss N. Altered central
48 sensorimotor processing in patients with complex regional pain syndrome.
49 *Pain* 2002;98:315–23.
50

- 1 22. Khalili N, Wendelschafer-Crabb G, Kennedy WR, Simone DA. Influence of
2 thermode size for detecting heat pain dysfunction in a capsaicin model of
3 epidermal nerve fiber loss. *Pain* 2001;91:241–50.
- 4
- 5 23. Kharkar S, Venkatesh YS, Grothusen JR, Rojas L, Schwartzman RJ. Skin
6 biopsy in complex regional pain syndrome: case series and literature
7 review. *Pain Physician* 2012;15:255–66.
- 8
- 9 24. Koban M, Leis S, Schultze-Mosgau S, Birklein F. Tissue hypoxia in
10 complex regional pain syndrome. *Pain* 2003;104:149–57.
- 11
- 12 25. Krämer HH, Eberle T, Uçeyler N, Wagner I, Klonschinsky T, Müller LP,
13 Sommer C, Birklein F. TNF- α in CRPS and “normal” trauma--significant
14 differences between tissue and serum. *Pain* 2011;152:285–90.
- 15
- 16 26. Lawless JF, Statistical models and methods for lifetime data. New York:
17 John Wiley & Sons Inc, 2003.
- 18
- 19 27. Lauria G, Lombardi R, Camozzi F, Devigili G. Skin biopsy for the diagnosis
20 of peripheral neuropathy. *Histopathology* 2009;54:273–85.
- 21
- 22 28. Legrain V, Guerit J, Bruyer R, Plaghki L. Attentional modulation of the
23 nociceptive processing into the human brain: selective spatial attention,
24 probability of stimulus occurrence, and target detection effects on laser
25 evoked potential. *Pain* 2002;99:21–39.
- 26
- 27 29. Legrain V, Perchet C, García-Larrea L. Involuntary orienting of attention to
28 nociceptive events: neural and behavioral signatures. *J Neurophysiol*
29 2009;102:2423–34.
- 30
- 31 30. Legrain V, Bultitude JH, De Paepe AL, Rossetti Y. Pain, body, and space:
32 what do patients with complex regional pain syndrome really neglect? *Pain*
33 2012;153:948–51
- 34
- 35 31. Maihofner C, Handwerker HO, Neundorfer B, Birklein F. Patterns of
36 cortical reorganization in complex regional pain syndrome. *Neurology*
37 2003;61:1707–15.
- 38
- 39 32. Maihofner C, Forster C, Birklein F, Neundorfer B, Handwerker HO. Brain
40 processing during mechanical hyperalgesia in complex regional pain
41 syndrome: a functional MRI study. *Pain* 2005;114:93–103.
- 42
- 43 33. Maihofner C, Seifert F, Markovic K. Complex regional pain syndromes:
44 new pathophysiological concepts and therapies. *Eur J Neurol*
45 2010;17:649–60.
- 46
- 47 34. Maihöfner C, Peltz E. CRPS, the parietal cortex and neurocognitive
48 dysfunction: an emerging triad. *Pain* 2011;152:1453–54.
- 49

- 1 35. Mouraux A, Ragé M, Bragard D, Plaghki L. Estimation of intraepidermal
2 fiber density by the detection rate of nociceptive laser stimuli in normal and
3 pathological conditions. *Neurophysiol Clin* 2012;42:281–91.
- 4
- 5 36. Oaklander AL, Rissmiller JG, Gelman LB, Zheng L, Chang Y, Gott R.
6 Evidence of focal small-fiber axonal degeneration in complex regional pain
7 syndrome-1 (reflex sympathetic dystrophy). *Pain* 2006;120:235–43.
- 8
- 9 37. Oaklander AL, Fields HL. Is reflex sympathetic dystrophy/complex regional
10 pain syndrome type I a small-fiber neuropathy? *Ann Neurol* 2009;65:629–
11 38.
- 12
- 13 38. Ochoa JL. Is CRPS I a neuropathic pain syndrome? *Pain* 2006;123:334–
14 35.
- 15
- 16 39. Ochoa J, Mair WG. The normal sural nerve in man. I. Ultrastructure and
17 numbers of fibres and cells. *Acta Neuropathologica* 1969;13:197–216.
- 18
- 19 40. Pleger B, Tegenthoff M, Schwenkreis P, Janssen F, Ragert P, Dinse HR,
20 Volker B, Zenz M, Maier C. Mean sustained pain levels are linked to
21 hemispherical side-to-side differences of primary somatosensory cortex in
22 the complex regional pain syndrome I. *Exp Brain Res* 2004;155:115–19.
- 23
- 24 41. Ragé M, Van Acker N, Facer P, Shenoy R, Knaapen MWM, Timmers M,
25 Streffer J, Anand P, Meert T, Plaghki L. The time course of CO₂ laser-
26 evoked responses and of skin nerve fibre markers after topical capsaicin in
27 human volunteers. *Clin Neurophysiol* 2010;121:1256–66.
- 28
- 29 42. Regan D. Human brain electrophysiology. Evoked potentials and evoked
30 magnetic fields in science and medicine. Amsterdam: Elsevier, 1989. pp.
31 53–55.
- 32
- 33 43. Reinersmann A, Haarmeyer GS, Blankenburg M, Frettlöh J, Krumova EK,
34 Ocklenburg S, Maier C. Left is where the L is right. Significantly delayed
35 reaction time in limb laterality recognition in both CRPS and phantom limb
36 pain patients. *Neurosci Lett* 2010;486:240–45.
- 37
- 38 44. Reinersmann A, Landwehr J, Krumova EK, Ocklenburg S, Güntürkün O,
39 Maier C. Impaired spatial body representation in complex regional pain
40 syndrome type 1 (CRPS I). *Pain* 2012;153:2174–81.
- 41
- 42 45. Schattschneider J, Hartung K, Stengel M, Ludwig J, Binder A, Wasner G,
43 Baron R. Endothelial dysfunction in cold type complex regional pain
44 syndrome. *Neurology* 2006;67:673–75.
- 45
- 46 46. Schmidt R, Schmelz M, Ringkamp M, Handwerker HO, Torebjörk HE.
47 Innervation territories of mechanically activated C nociceptor units in
48 human skin. *J Neurophysiol* 1997;78:2641–48.
- 49

- 1 47. Schulz E, Tiemann L, Schuster T, Gross J, Ploner M. Neurophysiological
2 coding of traits and states in the perception of pain. *Cereb Cortex*
3 2011;21:2408–14.
- 4
- 5 48. Stanton-Hicks M, Janig W, Hassenbusch S, Haddox JD, Boas R, Wilson P.
6 Reflex sympathetic dystrophy: changing concepts and taxonomy. *Pain*
7 1995;63:127–33.
- 8
- 9 49. Thimineur MA, Saberski L. Complex regional pain syndrome type I (RSD)
10 or peripheral mononeuropathy: a discussion of three cases. *Clin J Pain*
11 1996;12:145–50.
- 12
- 13 50. Thimineur M, Sood P, Kravitz E, Gudin J, Kitaj M. Central nervous system
14 abnormalities in complex regional pain syndrome (CRPS): clinical and
15 quantitative evidence of medullary dysfunction. *Clin J Pain* 1998;14:256–
16 67.
- 17
- 18 51. Treede RD, Kief S, Hölzer T, Bromm B. Late somatosensory evoked
19 cerebral potentials in response to cutaneous heat stimuli. *EEG Clin*
20 *Neurophysiol* 1988;70:429–41.
- 21
- 22 52. Treede RD, Lorenz J, Baumgärtner U. Clinical usefulness of laser-evoked
23 potentials. *Neurophysiol Clin* 2003;33:303–14.
- 24
- 25 53. Valentini E, Hu L, Chakrabarti B, Hu Y, Aglioti SM, Iannetti GD. The
26 primary somatosensory cortex largely contributes to the early part of the
27 cortical response elicited by nociceptive stimuli. *Neuroimage*
28 2012;59:1571–81.
- 29
- 30 54. Valeriani M, Pazzaglia C, Cruccu G, Truini A. Clinical usefulness of laser
31 evoked potentials. *Neurophysiol Clin* 2012;42:345–53.
- 32
- 33 55. van der Laan L, ter Laak HJ, Gabreels-Festen A, Gabreels F, Goris RJ.
34 Complex regional pain syndrome type I (RSD): pathology of skeletal
35 muscle and peripheral nerve. *Neurology* 1998;51:20–25.
- 36
- 37 56. Vartiainen NV, Kirveskari E, Forss N. Central processing of tactile and
38 nociceptive stimuli in complex regional pain syndrome. *Clin Neurophysiol*
39 2008;119:2380–88.
- 40
- 41 57. Walton KD, Dubois M, Llinas RR. Abnormal thalamocortical activity
42 inpatients with complex regional pain syndrome (CRPS) type I. *Pain*
43 2010;150:41–51.
- 44
- 45 58. Wu Q, García-Larrea L, Mertens P, Beschet A, Sindou M, Mauguière F.
46 Hyperalgesia with reduced laser evoked potentials in neuropathic pain.
47 *Pain* 1999;80:209–14.
- 48
- 49 59. Yarnitsky D, Simone DA, Dotson RM, Cline MA, Ochoa JL, Single C.
50 Nociceptor responses and psychophysical parameters of evoked pain:

- 1 effect of rate of rise of heat stimuli in humans. J Physiol (London)
- 2 1992;450:581–92.
- 3
- 4

FIGURE CAPTIONS

Figure 1. Average LEP waveform recorded at the vertex (Cz vs. A1-A2) after stimulation of the right and left foot dorsum of a female patient (30 years of age) presenting with CRPS-1 at the right foot for 2.8 years (severity score of 0.82). Note the increased latency and reduced amplitude of the N2 and P2 waves elicited by stimulation of the affected right foot (black waveform) as compared to the asymptomatic left foot (grey waveform). Scalp topographies show the distributions of the A δ -fiber related N2 and P2 waves elicited by stimulation of the asymptomatic left foot. The vertical dashed line represents the onset of the laser stimulus.

Figure 2. Average LEP waveforms from all patients with CRPS-1 in the lower limbs (feet dorsum stimulation) and with CRPS-1 in the upper limbs (hand dorsum stimulations). The individual LEPs obtained when stimulating the asymptomatic contralateral side (left panels) and the affected CRPS side (middle panels) are shown as superimposed thin grey waveforms. The group-level average is shown as a thick black line. The right panel shows the individual N2-P2 amplitudes of the LEPs obtained by stimulation of the contralateral and CRPS sides. Note that in the majority of patients, the N2-P2 amplitude of LEPs is reduced at the CRPS side as compared to the contralateral side.

Figure 3. Left panel. Group-level average LEP waveform and topographic maps at Cz (blue) and Pz (red) of all patients. Right panel. The statistic T value, revealed by the linear mixed model, shows an inverse and significant relationship between LEP amplitude and reaction times, maximum at Pz between 365 ms and 533 ms (grey area in both panels). On average, trials with larger P2 amplitudes were also trials with shorter reaction times. In contrast, the amplitude of the N1 and N2 components was not significantly correlated with the RTs. This may indicate that the variability of RTs could be mainly caused by cognitive influences.

Figure 4. Average LEP waveforms recorded at the vertex (C_z vs. A_1 - A_2) after stimulation of the right and left foot dorsum of a female patient (57 years of age) presenting with CRPS at the right limb for 12.7 years (severity score of 0.88). Stimulation of the asymptomatic left foot (grey waveform) clearly elicited two distinct negative-positive waves, the first with a latency compatible with the conduction velocity of A δ -fibers, the second with a latency compatible with the conduction velocity of C-fibers. Stimulation of the affected right foot evoked only elicited a LEP at a latency compatible with the conduction velocity of C-fibers. As shown by the scalp topographies of the N2 and P2 peaks, the topographical distribution of the C-fiber related LEP was well structured with a maximum at Cz-Pz. The lower part of the figure shows the time-frequency representation of LEP amplitude averaged across trials.