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Evidence of focal small-fiber axonal degeneration in complex regional pain syndrome-I (reflex sympathetic dystrophy)

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Abstract

CRPS-I consists of post-traumatic limb pain and autonomic abnormalities that continue despite apparent healing of inciting injuries. The cause of symptoms is unknown and objective findings are few, making diagnosis and treatment controversial, and research difficult. We tested the hypotheses that CRPS-I is caused by persistent minimal distal nerve injury (MDNI), specifically distal degeneration of small-diameter axons. These subserve pain and autonomic function. We studied 18 adults with IASP-defined CRPS-I affecting their arms or legs. We studied three sites on subjects' CRPS-affected and matching contralateral limb; the CRPS-affected site, and nearby unaffected ipsilateral and matching contralateral control sites. We performed quantitative mechanical and thermal sensory testing (QST) followed by quantitation of epidermal neurite densities within PGP9.5-immunolabeled skin biopsies. Seven adults with chronic leg pain, edema, disuse, and prior surgeries from trauma or osteoarthritis provided symptom-matched controls. CRPS-I subjects had representative histories and symptoms. Medical procedures were unexpectedly frequently associated with CRPS onset. QST revealed mechanical allodynia ($P < 0.03$) and heat-pain hyperalgesia ($P < 0.04$) at the CRPS-affected site. Axonal densities were highly correlated between subjects' ipsilateral and contralateral control sites ($r = 0.97$), but were diminished at the CRPS-affected sites of 17/18 subjects, on average by 29% ($P < 0.001$). Overall, control subjects had no painful-site neurite reductions ($P = 1.00$), suggesting that pain, disuse, or prior surgeries alone do not explain CRPS-associated neurite losses. These results support the hypothesis that CRPS-I is specifically associated with post-traumatic focal MDNI affecting nociceptive small-fibers. This type of nerve injury will remain undetected in most clinical settings.

Keywords: CRPS; RSD; Complex regional pain syndrome; Skin biopsy; PGP9.5; Human research

1. Introduction

CRPS is a baffling "pain plus" syndrome. Most cases follow trauma, but symptoms persist even after healing appears complete. Focal dysautonomia (vasomotor instability causing edema, skin color or temperature abnormalities, and/or hyperhydrosis) is required for diagnosis, and motor abnormalities are common (Schwartzman and Kerrigan, 1990). Some patients have mild or short-lived symptoms (Sandroni et al., 2003), but others are chronically disabled. CRPS was first recognized as *causalgia* ("burning pain") in wounded soldiers (Mitchell, 1872) and then after lesser injuries as reflex sympathetic dystrophy. In 1994, the IASP revised the taxonomy; "CRPS-I" classifies patients without known nerve injury, "CRPS-II" classifies those with nerve injuries (Merskey and Bogduk, 1994). CRPS-I is most common, with an annual U.S. incidence of 15,000 (Sandroni et al., 2003). The paucity of objective evidence of biological causes of CRPS-I has led some

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to doubt that one exists. Many cases are litigated, but it is hard to prove or disprove the presence of CRPS.

Several plausible hypotheses about pathogenesis circulate. One implicates incomplete healing with prolonged inflammation that sensitizes nearby neurons (Sudeck, 1901; Veldman et al., 1993; Weber et al., 2001). Identification of abnormal brain function engendered the hypothesis that CRPS originates there; variously in the medulla (Thimineur et al., 1998), thalamus (Rommel and Thimineur, 2001), or cortex (Schwenkreis et al., 2003). Others interpret CRPS-I as primarily psychiatric (Verdugo and Ochoa, 2000). We propose a simple hypothesis; that CRPS-I, like CRPS-II, is triggered by nerve injuries, those that are less severe and predominantly affect small-diameter axons (''small-fibers''). These may preferentially degenerate after trauma (Lekan et al., 1997).

Since symptoms of neurological injury reflect function of the neurons damaged, C- and/or A-δ fibers are obvious suspects because they mediate pain and autonomic function. Damage to them or to their central connections underlies all neuropathic pain (Oaklander, 1999). The identical symptoms and treatment responses of CRPS-I and CRPS-II (Calvillo et al., 1998; Hord and Oaklander, 2003) suggest a common cause. Furthermore, small-fiber polyneuropathies can cause widespread CRPS-like symptoms (Jeffcoate et al., 2004; Novak et al., 2001). Even CRPS-related focal osteopenia (Kozin et al., 1976) is explicable by our hypothesis because small-fibers regulate bone density (Hukkanen et al., 1993). Small-fiber neuropathies are notoriously cryptic because they lack familiar motor signs. Since small-fibers have no motor function, electromyography is insensitive and their small, slow action potentials are undetected by nerve conduction study.

We used the most sensitive method of detecting small-fiber damage – skin biopsy. Sections are immunolabeled against PGP9.5, a pan-neuronal marker, and axon density quantitated (Dalsgaard et al., 1989). Such biopsies have revealed neurite losses in every neuralgic condition studied (Griffin et al., 2001). Neurite losses are thought to cause pain by triggering hyperexcitability in remaining peripheral and central neurons (Woolf, 2004). The one previous skin-biopsy study of CRPS-I patients revealed no abnormalities (Drummond et al., 1996). Quantitative sensory testing (QST) was considered a secondary measure because it requires subject cooperation (Freeman et al., 2003) and yields subjective data. Previous QST studies of CRPS-I patients have identified varying abnormalities (Kemler et al., 2000; Sieweke et al., 1999).

2. Methods

2.1. Subjects

Adults were screened by telephone; enrollees consented to an IRB-approved protocol and completed an approved questionnaire about history and symptoms. Inclusion criteria were the presence of IASP-defined CRPS-I (Table 1) without specific characteristics, duration, or treatment history. We excluded candidates with impediments to consent, coagulopathy, use of blood-thinners, or potentially confounding neuropathic conditions, specifically diabetes, HIV, any neuropathy, consumption of more than two alcoholic beverages a day, spine surgery, or neurotoxin or chemotherapy exposure. Any history of regular topical capsaicin use, or any topical capsaicin use within the year before study mandated exclusion. CRPS patients with known nerve lesions identified by neurologic examination or electrophysiologic study were excluded as having CRPS-II. Study sites were chosen by having subjects identify their location of maximum pain and a nearby symptom-free spot (ipsilateral-control). A mirror-image contralateral-control site was also studied to detect and control for potential CRPS-related ipsilateral limb edema that might artifactually lower cutaneous neurite densities. All study sites were approximately equidistant from the spine.

The seven ''symptom-matched'' control subjects studied comprised five women and two men with clinically and radiographically documented knee osteoarthritis or traumatic injury leaving chronic pain, edema, disuse, and disability severe enough to require chronic pain medications, and arthroscopy or joint replacement. These subjects were studied with skin biopsy only at nearby painful and non-painful sites within their affected limb to investigate whether CRPS-associated epidermal neurite losses might be incidental sequelae of chronic limb pain, edema, disuse, or prior limb trauma or surgery. Most subjects were studied shortly after knee joint replacement. Despite having similar symptoms, the control subjects did not meet the criteria for CRPS because they had objective evidence of current tissue injury proportionate to and consistent with their pain and other symptoms. Furthermore, they lacked the skin color changes and hyperhydrosis documented to differentiate CRPS from acute tissue injury and inflammation (Birklein et al., 2001).

2.2. Functional testing

Fewer subjects (13) were studied with QST than skin biopsy because a thermal generator was unavailable at study onset. After its purchase, all subsequent consenting subjects

Table 1
Diagnostic criteria for CRPS-I (reflex sympathetic dystrophy) according to the International Association for the Study of Pain (IASP)

1. The presence of an initiating noxious event, or a cause of immobilization.
2. Continuing pain, allodynia, or hyperalgesia with which the pain is disproportionate to any inciting event.
3. Evidence at some time of edema, changes in skin blood flow, or abnormal sudomotor activity in the region of the pain.
4. This diagnosis is excluded by the existence of conditions that would otherwise account for the degree of pain and dysfunction.

Note. Criteria 2–4 must be satisfied (Merskey and Bogduk, 1994).

were also studied by QST. To quantitate mechano-sensation, subjects rated the presence and severity (where 0 = no pain and 10 = worst pain imaginable) of pain induced by stroking with a small paintbrush. Pressure was evaluated using a thermoneutral rod and mechanical hyperalgesia by safety-pin touch. Response to brush, rod, and pin were coded "abnormal" if undetected or painful, and "normal" if detected without pain. To quantitate thresholds for detection of punctate mechanical stimuli, graded von Frey monofilaments (Stoelting, Wood Dale, IL) were applied using a staircase protocol, where change in response for two consecutive filaments defined threshold boundaries (Cornsweet, 1962). The geometric mean of boundary forces was calculated, and the median of three repetitions was used for analysis. Thresholds for warmth, cool, heat-pain, and cold-pain were detected using a Peltier generator (TSA 2001; Medoc, Ramat Yishai, Israel) and the method of limits (Gescheider, 1997). Thermode area was 3 cm^2, starting temperature was 32 °C, ramp rates were 1 °C/s for detection thresholds and 1.5 °C/s for thermal-pain thresholds, and safety cutoffs were 50 and 0 °C. Thermal-perception thresholds were measured four times and thermal-pain thresholds thrice; the arithmetic means of these repeated measures defined threshold.

2.3. Anatomical testing

After QST and subcutaneous administration of local anesthesia, one 3-mm diameter skin punch per study site was removed, fixed, and cryoprotected using standard methods (Amato and Oaklander, 2004). There was no serious adverse event and no subject reported worsening of CRPS symptoms. One subject developed inflammation at one biopsy site that was additionally traumatized by her dressing; this resolved completely within 1 day of rebandaging and administration of oral antibiotics.

Punches were sectioned vertically and four sections were chosen by systematic-random sampling to be immunolabeled against PGP9.5 (Chemicon, Temecula, CA) using standard methods (Amato and Oaklander, 2004). Primary antibody omission produced labeling failure. Localization of epidermal PGP9.5 immunolabeling to axons has been ultrastructurally verified (Hilliges et al., 1995). Quantitative data can only be obtained from the epidermis where axons individuate. Almost all PGP9.5 immunoreactive epidermal neurites have been identified as $TRPV1^+$ nociceptors (Simone et al., 1998). Slides were masked and randomized, then all epidermal $PGP9.5^+$ neurites were quantitated by a single skilled morphometrist using standard methods (Amato and Oaklander, 2004). Our laboratory reports neurite densities per mm^2 skin surface area rather than per linear mm as some other groups do to factor in the thickness of the tissue sections studied.

2.4. Statistical analysis

Non-parametric analyses were used because small sample sizes precluded determination of normality. One site (the contralateral-control) had not been studied in the first subject (#9). Continuous sensory testing data from different sites within the same subject were compared using Wilcoxon's signed rank tests. Dichotomous data were compared by McNemar test. Inter-variable relationships were evaluated by Spearman's correlations. Statistical significance represented $P \leqslant 0.05$. SAS 9.1 software (SAS Institute, Inc., Cary, NC) was used.

3. Results

3.1. Characteristics of subjects

Table 2 summarizes the demographic and clinical features of the 18 CRPS-I subjects. Mean subject age was 38.5 ± 11.3 years. All but one were Caucasian. Right and left sides were randomly affected. Nine subjects were disabled, seven were employed and two were students. Medical procedures were common at CRPS onset. Ten subjects reported that their CRPS-inducing trauma was almost immediately treated with surgery and/or casting, and they were unsure of the relative causal contributions of trauma versus treatment. Four subjects had no accidental trauma, only medical procedures (usually elective surgery) just preceding CRPS onset. Two subjects had delayed surgical treatment of a traumatic injury and were certain that these surgeries rather than their earlier traumas had caused the onset of their CRPS symptoms. Only two subjects had not undergone any medical procedures just before CRPS onset.

The symptoms that CRPS subjects identified using a questionnaire (Table 3) appeared representative of those described in larger samples (Veldman et al., 1993). Using a 0–10 scale, the mean severity of overall pain was 6.5 ± 2.4, of pain while at rest (stimulus-independent pain) was 6.8 ± 2.3, of pain elicited by touch (mechanical allodynia) was 5.6 ± 3.7, severity of sharp, shooting, lancinating pain was 8.3 ± 2.5. All subjects reported experiencing current or previous edema in their CRPS-affected area, 67% reported current or previous numbness, 94% reported current or previous temperature and/or color changes, 94% reported current or previous weakness, and 59% reported current or previous changes in sweating. Eighty-nine percent of subjects were using pain medications at the time of study (Table 2) and 83% had undergone procedures to try and reduce pain, most commonly sympathetic blocks. Four subjects reported trying topical capsaicin for their symptoms, none used it regularly because it was too painful, and none had applied it within a year before study. No subjects with severe limb edema or trophic changes applied for enrollment in this study.

3.2. Functional testing

Quantitative sensory testing (Table 4) did not reveal statistically significant differences between the CRPS-affected and contralateral-control site for perception of cool or warmth, or for detection of cold pain. Heat-pain hyperalgesia ($P = 0.039$) and static punctate mechanical allodynia (to von Frey filaments) ($P = 0.032$) were

Table 2
Demographic and clinical data about subjects with CRPS-I

Subject ID	Age (years)	Sex	CRPS duration (years)	Limb	Potential contributors to CRPS			Treatments				
					Events before CRPS onset	Accidental trauma	Medical procedures	Medications used at time of study				Procedures, current or previous
								Opioids	Tricyclics	Anti-epileptic	Other related	
1	45	Female	2.3	Arm	Medical	None	Removal benign axillary tumor	Oxycodone	Nortriptyline		Lorazepam	Neural block
2	47	Male	4.1	Leg	Fall at work	FX-calcaneous	ORIF, bivalve cast	Tramadol		DPH		Neural block
3	20	Female	6.5	Leg	Sports	Soft tissue injury	Surgery, decasted for postop. hematoma	Oxycod., hydrocod.		Oxcarbazepine		TENS, ESI, AP, hyperbaric O2, bretyllium block
4	30	Female	6.0	Arm	Medical	None	Carpal tunnel release	Morphine			Flexeril	Removal of rib for thoracic outlet syndrome
5	55	Male	1.4	Leg	MVA	FX-navicular	Long leg cast		Nortriptyline	GBP	Ibuprofen	Neural block, TENS
6	31	Female	4.3	Leg	Unclear	Patellar dislocat.	Several knee surgeries	Oxycodone	Desipramine	GBP, CMZ	Ibuprofen	Neural block, stimulator
7	54	Male	2.5	Leg	Medical	None	Vascular-saphenous bypass graft		Desipramine			Neural block
8	41	Female	2.9	Leg	MVA	FX-tibia, fibula	ORIF	Oxycodone	Nortriptyline	GBP	Lidocaine patch	Neural block, removal of orthopedic implants
9	32	Female	0.9	Leg	Sports	Soft tissue injury	Knee surgery, artery cut, compartment synd., fasciotomy		Nortriptyline	GBP		Neural block
10	35	Female	3.2	Arm	Medical	None	Traumatic phlebotomy			GBP	Prescribed antiperspirant	
11	44	Male	2.1	Leg	Work	Soft tissue injury	None					
12	33	Female	0.4	Leg	Sports	Soft tissue injury	Knee surgery	Methadone		GBP	Lidocaine patch	Neural block
13	41	Female	18.2	Leg	MVA	Soft tissue injury	Surgery 5 years after MVA caused CRPS symptoms	Methadone	Desipramine			Neural block, TENS, surgical sympathectomy
14	39	Male	4.3	Leg	Fall	FX-tibia, fibula	ORIF	Tramadol				Neural block
15	45	Male	1.2	Arm	Sports	FX-radius	Casted, very painful, urgently uncasted		Nortriptyline			Neural block, carpal tunnel release
16	26	Male	2.4	Leg	MVA at work	Soft tissue injury	None	Oxycodone			Naprosyn	Neural block
17	18	Female	3.7	Leg	Unclear	Soft tissue injury	Fasciotomy × 2 for compartment synd.	Oxycodone				Neural block, TENS, neurolysis
18	57	Female	8.3	Arm	MVA	FX ribs and arm	ORIF and bone grafting				Ibuprofen	

MVA, motor vehicle accident; FX, fracture; ORIF, open reduction, internal fixation; CMZ, carbamazepine; DPH, diphenylhydantoin; GBP, gabapentin; AP, acupuncture; TENS, transcutaneous electrical neural stimulation; ESI, epidural steroid injection.

Table 3
Prevalence of CRPS symptoms, and severity of pain in CRPS subjects, as assessed by questionnaire

Subject ID	Sensory symptoms in affected limb (0–10)					Autonomic and motor symptoms in affected limb (0–10)			
	Numbness	Overall pain score	Pain while at rest	Touch allodynia	Lancinating pain	Limb edema	Color or temperature changes	Sweating changes	Weakness
1	Yes	6.5	6.5	4.0	7.0	Yes	Yes	Yes	Yes
2	No	3.5	3.5	0.0	8.0	Yes	Yes	Yes	No
3	No	9.0	7.5	7.5	9.0	Yes	Yes		Yes
4	Yes	9.0	9.0	10.0	10.0	Yes	Yes	Yes	Yes
5	No	5.0	6.0	3.0	6.0	Yes	Yes	No	Yes
6	Yes	8.0	8.0	6.0	10.0	Yes	No	No	Yes
7	No	8.0	8.0	8.0	0.0	Yes	Yes	No	Yes
8	Yes	4.5	4.5	7.0	9.0	Yes	Yes	No	Yes
9	Yes	4.0	5.5	0.0	9.5	Yes	Yes	Yes	Yes
10	Yes	8.0	8.0	8.0	10.0	Yes	Yes	Yes	Yes
11	Yes	4.0	4.0	0.0	8.0	Yes	Yes	No	Yes
12	No	8.0	8.0	10.0	10.0	Yes	Yes	Yes	Yes
13	Yes	7.5	6.5	0.0	9.0	Yes	Yes	Yes	Yes
14	No	1.0	1.0	3.0	5.0	Yes	Yes	Yes	Yes
15	Yes	10.0	9.0	10.0	10.0	Yes	Yes	Yes	Yes
16	Yes	9.0	9.0	9.0	9.0	Yes	Yes	No	Yes
17	Yes	5.0	10.0	7.5	10.0	Yes	Yes	No	Yes
18	Yes	6.8	8.0	8.0	9.5	Yes	Yes	Yes	Yes

Table 4
Results of quantitative sensory testing

	Contralateral control site	Ipsilateral control site	CRPS-affected site
Thermal thresholds (°C)			
Perception of cooling	28.8 (26.3–29.5)	28.4 (26.2–30.2)	25.1 (24.8–27.7)
Perception of warming	40.1 (38.2–47.1)	38.5 (35.8–43.6)	41.5 (35.5–45.8)
Perception of heat pain	49.1 (48.0–50.0)	48.1 (44.5–49.8)	46.7 (39.1–50.0)
Perception of cold pain	8.5 (0.0–24.3)	5.9 (0.0–24.1)	13.4 (0.6–22.7)
Mechanical			
Threshold for von Frey detection (mN)	3.6 (0.7–8.9)	6.0 (2.1–16.0)	8.9 (6.0–18.3)
Number of subjects with pain elicited by pinprick (0–10) (mechanical hyperalgesia)	1	0	5
Number of subjects with pain elicited by brushing (0–10) (mechanical allodynia)	0	0	4
Number of subjects with pain elicited by pressure (0–10)	0	0	2

For thermal and von Frey testing, the median values and the range between the upper and lower quartile are given. Statistically significant differences were present only at the CRPS-affected site for heat-pain hyperalgesia and mechanical allodynia.

present at the CRPS-affected site only. Perceptions of pin, brush, and/or pressure were occasionally abnormal at the CRPS-affected site.

3.3. Anatomical testing

3.3.1. Skin-biopsy data from control sites

Evaluation of skin-biopsy data (Fig. 1) from CRPS-I subject's unaffected control sites revealed considerable between-subject variability (range = 31–1702 neurites/mm^2 skin surface area), but high within-subject, between-site correlation (Spearman's $r = 0.97$), which confirmed the lack of intrinsic between-limb variability in epidermal innervation. Comparison of skin-biopsy data from CRPS-I subjects' ipsilateral- and contralateral-control sites was used to quantitate the effects of potential limb edema upon density of epidermal innervation. Based on clinical experience, we assumed that edema would be equivalent at the two nearby study sites on the CRPS-affected limb (the ipsilateral-control and CRPS-affected sites) and that CRPS-related edema would not affect the limb contralateral to the site of injury and CRPS. Thirteen subjects had higher neurite densities at the contralateral-control site, four subjects had higher densities at the ipsilateral-control site. The median density at the ipsilateral-control site was 348 neurites/mm^2 skin surface area and that at the contralateral-control site was 367 neurites/mm^2 skin surface area. Neurite densities at the ipsilateral-control site averaged $95 \pm 10\%$ of densities at the contralateral-control site

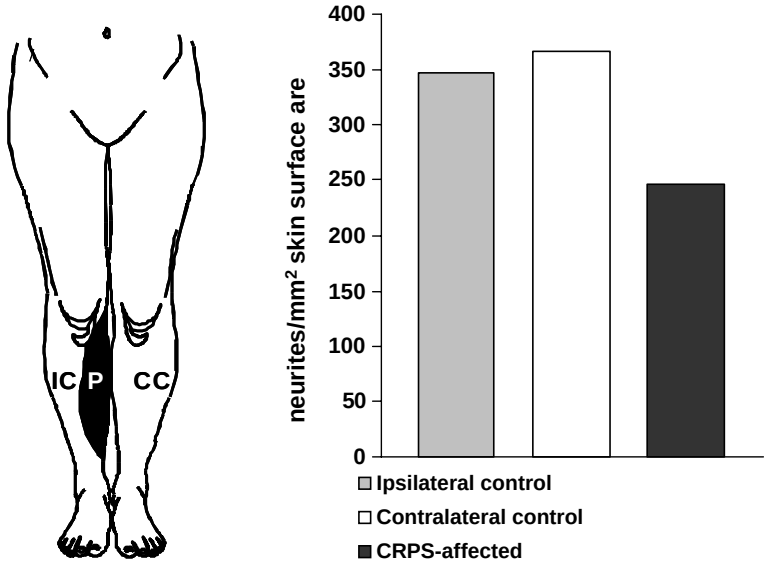

Fig. 1. Graphical representation of the relative locations of typical study sites ("P" represents the painful CRPS-affected site, "IC" the ipsilateral control site, and "CC" the contralateral control site). The bar graph illustrates data from PGP9.5-immunolabeled skin biopsies. Because data are collected from different sites on the limbs, and these have different neurite density norms, no measures of variability are given. Neurite densities from the CRPS-affected site are significantly reduced.

($P = 0.347$) suggesting that, on average, although some CRPS-limb edema may have been present, its overall magnitude was mild at the time of study.

3.3.2. Skin-biopsy data from the CRPS-affected site

Densities from the CRPS-affected site were significantly reduced to a median of 247 neurites/mm^2 skin surface area. This represented $71 \pm 15\%$ of values from the contralateral-control-site ($P < 0.001$) and $78 \pm 18\%$ of ipsilateral-control-site values ($P < 0.001$) (Fig. 1). Within the control group of subjects with chronic non-CRPS osteoarthritis pain and orthopedic limb surgery, median neurite densities from painful and control sites were not different ($P = 1.00$).

4. Discussion

Quantitation of epidermal innervation at painful CRPS-affected and nearby non-painful control sites has revealed focal CRPS-associated losses averaging 1/4 of neurites. Because CRPS-associated edema might have artifactually lowered neurite densities, we compared innervation densities at control sites on two separate limbs. This demonstrated that CRPS-associated limb edema causes only a small part of observed neurite reductions, leaving most of it best explained by CRPS-I nerve damage. Given that every subject reported affected-limb edema (Table 3), we expected, but did not find, significant edema-related neurite density reductions at the ipsilateral-control site. This discrepancy may be explained by the recent demonstration of CRPS-I somatosensory delusions of affected-limb edema. Their magnitude is independent of measured edema and correlates with CRPS duration (Moseley, 2005). They are attributed to somatosensory cortex plasticity.

Our methods permit quantitation of only the small-diameter unmyelinated (C-fiber) and thinly myelinated A-δ fibers that transmit nociceptive information and have autonomic efferent and trophic activities. Several previous studies have demonstrated enhanced vasodilation (Weber et al., 2001; Leis et al., 2004) in CRPS patients' limbs. In order to investigate whether neurite losses might be a consequence of CRPS symptoms rather than a cause, neurite densities were also measured in symptom-matched non-CRPS subjects; those data suggest that neither limb pain, swelling, disuse, nor prior surgery alone is sufficient to explain CRPS-associated neurite losses.

The major limitation of our study was size. All small studies risk inaccurate representation of the population of interest. This is even more a concern with ill-defined diseases. However, recruitment of CRPS subjects for even minimally invasive procedures is difficult. The one previous skin-biopsy study evaluated nine CRPS-I subjects, far fewer than those studied here (Drummond et al., 1996). We based subject classification on the best criteria available, the current IASP consensus standards (Table 1), and were careful not to restrict inclusion to specific patient subtypes, another potential source of bias. Because most subjects were hospital outpatients, we considered the possibility that they might have more severe disease than CRPS-affected individuals in the community (Sandroni et al., 2003), but it is not inappropriate for proof-of-concept studies to focus on severely affected patients. There is also a converse possibility; that more severely affected patients would

be disproportionately reluctant to be studied and thus under-represented. This may have occurred, since none of our subjects had gross edema or trophic changes. Tables 2 and 3 demonstrate that our sample is comparable to those published by other CRPS investigators.

The small size of our study limited the analysis. There were too few subjects to evaluate correlations between neurite densities, demographics, or the presence or severity of specific symptoms, demonstrating need for a larger skin-biopsy study with greater power. Longitudinal information became available when one subject (#12) with recent CRPS, severe pain, and profound neurite loss (to 3% of control) requested restudy 9 months later due to near-resolution of symptoms. Repeat biopsies taken just proximal to the first ones revealed dramatically increased neurite densities at her CRPS-affected site (to 95% of control), raising the question of whether recovery from CRPS is associated with successful axonal regeneration.

The range of neurite densities from unaffected-control sites was higher than expected. The high correlation of neurite densities from subject's ipsilateral and contralateral control sites indicates that this is mostly between-subject variability, due partly to normal differences in innervation density of the different body regions studied in different subjects (Lauria et al., 1999). To control for between-region variability, we used within-region control sites rather than published norms for the leg (McArthur et al., 1998). It is also possible, but not tested here, that some CRPS-I patients have globally abnormal neurite densities. Both unusually high and low cutaneous neurite densities are associated with polyneuropathy [early on, axonal fragmentation can artifactually elevate neurite counts (Amato and Oaklander, 2004)], but it is unexplored whether or not CRPS is associated with subclinical polyneuropathy. There is limited evidence that subclinical polyneuropathy increases risk for a different focal neuralgia, postherpetic neuralgia (PHN) (Baron et al., 1997), so further investigation may be indicated.

The variability and overall minimal severity of CRPS-related focal neurite losses helps explain why the previous well-designed skin-biopsy study of CRPS-I did not detect them (Drummond et al., 1996). Qualitative visual inspection was used, rather than stereologic quantitation as here, but inspection alone is insensitive to subtle cell losses. The only previous quantitative anatomical study of CRPS nerves that we identified drew conclusions congruent with ours. van der Laan and colleagues (1998) examined nerves from the amputated legs of eight severely affected CRPS-I patients. Light microscopy showed no major abnormalities of myelinated fibers. Ultrastructural quantitation of fiber spectra, necessary to evaluate unmyelinated fibers, could only be performed in the sural nerves, the only nerve for which normative values were available. Although their subjects' CRPS-inducing injuries had affected various parts of their legs, four of the eight legs had loss of sural C-fibers, of mild-moderate severity. These important pathological data from a more-proximal location within CRPS-affected nerves therefore complement our distal data. Our QST results are congruent with larger studies of CRPS-I that identified abnormalities consistent with sensory axonopathies and the central changes that these can evoke (Birklein et al., 2000; Kemler et al., 2000; Sieweke et al., 1999). Thus, our secondary outcome measure provides additional support for our hypothesis that CRPS-I patients have focal small-fiber axonal losses.

The identification of small-fiber damage in CRPS does not preclude involvement of other peripheral neurons. Most nerves contain mixed motor, sensory, and post-ganglionic sympathetic axons, and an individual nerve-injury patient's symptoms will reflect the types of axons injured. CRPS patients with additional motor axon damage may be more likely to have motor symptoms or abnormal electrophysiological test results, and thus to be classified as CRPS-II. Other tissues, such as blood vessels, bones and joints, and muscles, can become abnormal in CRPS and contribute to symptoms. It is unknown which of these other changes are initiated by small-fiber injury. Similarly, our methods do not allow us to study the secondary CNS abnormalities that contribute to CRPS. Since very few of the many people who sustain minimal distal nerve injuries develop CRPS, MDNI may be necessary but not sufficient to cause CRPS. Perhaps CRPS only ensues in nerve-injury patients who sustain an additional "second hit", such as development of specific secondary CNS abnormalities. Simultaneous investigation of CRPS subjects' PNS and CNS abnormalities (e.g., by simultaneous skin biopsy and functional imaging) might address this question.

Our small study permitted detailed review of histories, which suggested that medical procedures might initiate CRPS-I more often than currently appreciated. Most CRPS investigators collect data about the accidental traumas preceding CRPS onset, but few document the additional trauma caused by treatment of those injuries. For instance, knee sprains can lead to arthroscopy, which leaves up to 1/4 of patients with trochar injury to the infrapatellar branch of the saphenous, a pure sensory nerve (Mochida and Kikuchi, 1995). Some of these nerve-injured patients will be left with chronic lower-leg pain, including CRPS (Table 2). Inadequate consideration of medical procedures might contribute to the impression that CRPS-I often follows trivial injuries.

The methods used here do not appear promising for routine clinical diagnosis. QST is subjective and laborious, and no diagnostically useful pattern has emerged from larger studies (Birklein et al., 2000). Our data suggest that neurodiagnostic skin biopsy (also laborious and expensive) is also impractical because of the variable

and often minimal severity of axonal losses. Skin biopsies are only considered diagnostic of small-fiber polyneuropathy when neurite densities are below the 5th centile of population norms (McArthur et al., 1998), far more drastic losses than those of most of our CRPS subjects. Perhaps surrogate markers of small-fiber axonopathy such as vasodisregulation (Wasner et al., 2002) or abnormal sweating (Sandroni et al., 1998) will prove diagnostically useful.

Comparison of our CRPS results with those of a skin-biopsy study of patients with or without PHN after shingles shows neurite losses in PHN-affected skin to be far worse, averaging 80% (Oaklander et al., 1998). PHN develops almost exclusively in shingles patients with focal losses of more than 2/3 of epidermal innervation (Oaklander, 2001), which led us to hypothesize that drastic reduction of incoming signals from primary nociceptive neurons to CNS targets (deafferentation) is important in PHN pain pathogenesis. The same does not appear true in CRPS-I, where abnormal activity of remaining primary afferents may play a larger role.

The major implications of our results are theoretical. They support the concept that CRPS-I is a neurological condition, and that small-fiber axonal damage is involved in pathogenesis. They unify the CRPS-I and CRPS-II subtypes. They challenge other views that CRPS-I arises exclusively in the brain, or is a"pseudo-neurological" illness cultivated consciously for secondary gain, or unconsciously by psychiatric patients with somatization or conversion disorder. They imply that evaluation of CRPS-I patients by peripheral nerve specialists would help identify their lesions, and that therapies effective for neuropathic pain (neuralgia) are appropriate for treatment of CRPS-I. We hope that our findings reduce confusion about this syndrome and improve clinical care and research.

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