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Ai miei bambini
Giorgio, Giovanni e Anita

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Introduction

Pain usually develops when tissue-damaging stimuli activate peripheral nociceptive afferents.

Neuropathic pain arises by activity generated within the nervous system without adequate stimulation of its peripheral sensory endings. A widely accepted definition of neuropathic pain is "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" [1].

Neuropathic pain is a frequent problem in many peripheral and central nervous system (CNS) diseases. The peripheral nerve diseases that most commonly cause neuropathic pain are distal symmetric peripheral neuropathies (e.g. diabetic neuropathy), and focal neuropathies related to trauma (e.g. traumatic brachial plexus injuries) and to surgical interventions (e.g. breast surgery). Exemplary CNS diseases causing neuropathic pain include multiple sclerosis, spinal cord injury and stroke. The widely ranging etiologies suggest a high prevalence of neuropathic pain in the general population. [2]

Neuropathic pain typically manifests with continuous pain (burning, squeezing, pressure) or paroxysmal pain (electric shock-like sensations, stabbing pain), and provoked (brush-evoked, pressure-evoked, cold-evoked), or paraesthetic and dysaesthesic (tingling, pins and needles) sensations. In a study of 482 patients with neuropathic pain, the three most frequently reported pains were ongoing burning pain (65.4%), paroxysmal electric shock-like pain (57.0%), and brush-evoked pain (54.9%), with most patients reporting coexistence of heterogeneous sensory signs and symptoms.[3]

Clinicians in the field generally agree that patients with neuropathic pain that seems to be refractory to treatment may nevertheless

have treatable disease, emphasizing the need for careful diagnostic assessment.[4] In recent years, increased combination of newly proposed screening questionnaires, and diagnostic procedures such as quantitative sensory testing (QST), pain-related evoked potentials and skin biopsy, have advanced the mechanistic approach to pain management, leading to the development of so-called sensory profiles (that is, different combinations of pain-related sensory abnormalities)[5][6][7].

Convincing evidence on the relationship between the underlying pathophysiological mechanisms and neuropathic pain symptoms now suggests that classifying neuropathic pain according to a mechanism-based rather than an etiology-based approach might help in targeting therapy to the individual patient and would also be useful in testing new drugs. [8][9][10] In the next pages I will resume the work by our group in clarifying how symptoms translate into mechanisms, with a focus on the findings about the role of large myelinated fibres in mediating paroxysmal pain.

1. Types of neuropathic pain

1.1. Ongoing burning pain

Although no pathognomonic symptom of neuropathic pain exists, the most typical type of pain in this condition is burning pain. Clinical studies report that the frequency of burning pain ranges from 51% to 90% in patients with peripheral neuropathy.[11]

QST shows at least one 'sensory' abnormality in almost all patients with neuropathic pain, but no studies have defined a clear relationship between ongoing burning pain and specific sensory abnormalities. Conversely, neurophysiological studies have shown that in patients with neuropathic pain related to peripheral and CNS diseases (postherpetic neuralgia, carpal tunnel syndrome, pain is inversely related to LEP amplitude. This relationship—although only an indirect finding in some instances—indicates that ongoing burning pain is closely associated with damage to the nociceptive system.[8][9][10]

Depending on whether the distal and proximal nerve terminals are anatomically spared, four mechanisms could, in theory, be responsible for ongoing burning pain. The first mechanism applies to conditions in which most distal terminals are spared and nociceptors are

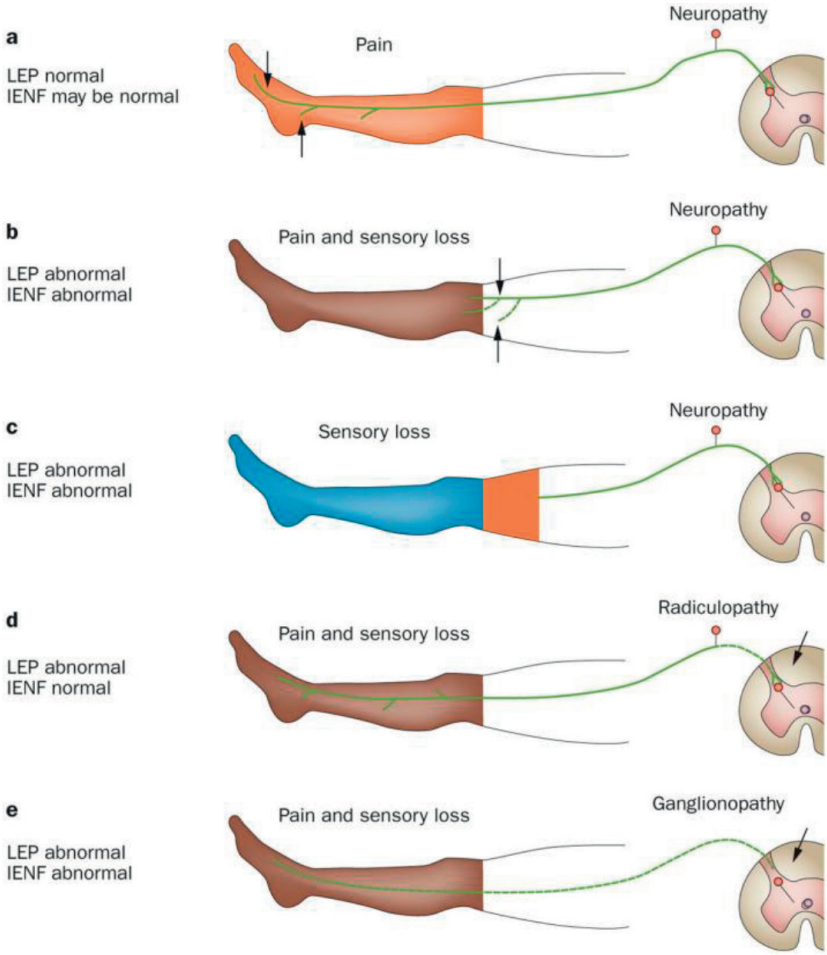


Fig.1. Models for ongoing burning pain in primary sensory neuron disease. Arrows indicate pain source. Central sensitization can develop in these conditions. **a** | Initially, pain can be generated by peripheral sensitization ('irritable nociceptors'). **b** | In length-dependent polyneuropathy with continuous nerve regeneration, pain from regenerating sprouts is felt in the same territory of thermal-pain hypoaesthesia. **c** | In length-dependent polyneuropathy without regeneration, pain is absent in the area of thermal-pain hypoaesthesia. Peripheral sensitization can cause pain in the immediately proximal region. **d** | In postganglionic lesions that cause anatomical denervation of second-order neurons, pain is felt in the same territory as thermal-pain sensory loss, but the distal axon does not degenerate. **e** | When the cell body of the primary sensory neurons dies, proximal and distal portions of the axon degenerate. Pain is generated by the second-order neuron and is felt in the same territory as thermal-pain hypoaesthesia. Abbreviations: IENF, intraepidermal nerve fibre; LEP, laser-evoked potential.

sensitized (termed 'irritable' nociceptors). The second mechanism is based on so-called 'regenerating sprouts': in polyneuropathy that induces axon-length-dependent degeneration together with continuous

regeneration attempts, newly generated nerve sprouts are hyperexcitable.[12] The third mechanism, which is termed 'functional deafferentation', refers to distal axonal degeneration that is unaccompanied by functionally important regeneration; in such circumstances, second-order neurons in the spinal cord, though still anatomically connected to the primary afferents, lose their physiological input. Finally 'anatomical denervation' refers to processes such as ganglionopathy or root lesion, in which proximal terminals degenerate and presynaptic boutons drop, thereby exposing the vacated postsynaptic membrane (Fig. 1).

1.2. Electrical shock-like pain

Established knowledge postulates that neuropathic pain invariably arises from nociceptive pathway damage. The underlying rationale hinges on the obvious reasoning that pain pathway dysfunction will produce painful sensations and on clinical and experimental evidence documenting pain pathway damage.[13] This assumption receives no support, however from neurophysiological studies conducted in our laboratory over recent years.[8][9][10] Our neurophysiological studies showed that in peripheral nervous system diseases, paroxysmal electrical shock-like pain is associated with evidence of large nonnociceptive myelinated fiber pathway damage. In patients with postherpetic neuralgia and carpal tunnel syndrome, paroxysmal pain is associated with abnormalities involving non nociceptive Aβ fibers. This correlation per se does not necessary imply a direct causative association; nevertheless, the relationship between electrical shock-like pain and large myelinated fiber demyelination indirectly receives support from numerous observations in trigeminal neuralgia, the neuropathic pain condition most typically causing paroxysmal electrical shock-like pain.[14] In idiopathic trigeminal neuralgia (caused by vascular compression on the trigeminal root by tortuous or aberrant vessels) and symptomatic trigeminal neuralgia because of a tumour compressing the trigeminal nerve root, demyelination caused by the focal compression mainly damages large myelinated fibers. This pathological mechanism agrees with animal studies showing that a pneumatic cuff compressing the peripheral nerve affected large myelinated fibers but left small myelinated and unmyelinated fibers unaffected. [15] Symptomatic trigeminal neuralgia due to a pontine demyelinating plaque

related to multiple sclerosis invariably arises from demyelination involving primary afferent fibers.[16] Whether produced by multiple sclerosis or chronic compression, Aβ-fiber demyelination increases neuronal susceptibility to ectopic excitation and high-frequency discharges, producing typical paroxysmal electrical shock-like pain.[17] The proposed mechanism for electric shock-like pain indicates as the most appropriate treatments carbamazepine and oxcarbazepine. Insofar as such agents produce a frequency- dependent voltage-gated sodium channel block and, thereby, reduce action potential firing frequencies. That focal demyelination can induce bursts of high-frequency discharges according with neurophysiological evidence in patients with hemifacial spasm. Hemifacial spasm is caused by a neurovascular conflict between an aberrant vessel and the facial nerve, causing focal demyelination involving the facial nerve root.[18]

Facial muscle electromyography shows that the tonic facial muscle contraction in hemifacial spasm corresponds to highfrequency motor unit impulses that can reach up to 200 Hz. [19] Hence, the observation that focal demyelination involving large myelinated motor axons produces a paroxysmal movement disorder which implies that focal demyelination affecting large myelinated sensory nerve fibers produces paroxysmal electrical shock-like pain. Several animal studies describing spontaneous ectopic discharges recorded in Aβ-fiber axons after nerve injuries support human clinical investigations.[20]

In animal models of peripheral neuropathy, the axonal population in which ectopic activity develops predominantly and early is the large myelinated fibers.[21] In animal models of multiple sclerosis, intraxonal recordings from demyelinated axons in the dorsal columns show abnormal spontaneous activity consisting of evenly spaced impulses at frequencies of 10 to 50 Hz and high-frequency bursts lasting up to 5 seconds.[22]

Although human and animal evidence supports the idea that paroxysmal electrical shock-like pain is related to Aβ-fiber damage, opinions differ on whether the high-frequency bursts in focally demyelinated Aβ fibers are sufficient to provoke pain per se. High-frequency bursts activating large fiber terminals can partially depolarize adjacent C-fiber terminals, but the mechanisms responsible for transferring Aβ-fiber activity to non-myelinated axons remain controversial. They might act by crossing depolarizing afferent terminals in the dorsal horn, either through collateral axono-axonal synapses or through interneuron. [23] A further possibility is that the high-frequency discharge in

damaged Aβ fibers reaches the wide dynamic range (WDR) neurons, where it mimics the painful input. The WDR neurons lie in lamina V in the dorsal horn and receive both large and small fiber terminals. They can distinguish and project nociceptive and non nociceptive sensory information, encoding neuronal firing rates (higher for noxious and lower for non noxious stimuli). We propose that when the ectopically generated, high-frequency discharge in damaged Aβ fibers reaches the WDR neurons, it mimics the painful input, even in unsensitised WDR neurons. Hence, an afferent input originating in non nociceptive fibers can spread to nociceptive pathways, thus producing pain.

1.3. Dynamic mechanical allodynia

Provoked pain includes pain evoked by various types of stimuli. Allodynia is the preferred term for describing pain evoked by normally non painful stimuli. Warm and cold allodynia indicate pain due to normally non painful warm and cold stimuli. Mechanical static allodynia refers to pain from normally non painful static pressure stimuli on the skin; conversely, dynamic mechanical allodynia indicates pain evoked by light tactile stimuli.[24] The most common type of provoked pain is dynamic mechanical allodynia (hereafter, simply termed allodynia). Its prevalence on patients with neuropathic pain ranges from 18% to 55%.[24] Typical clinical observations include patients with thoracic postherpetic neuralgia reporting pain due to the contact between skin and shirt, and patients with diabetic neuropathy reporting pain due to the contact between feet and bed sheets.

No general agreement exists about the pathophysiological mechanism underlying allodynia in patients with peripheral nervous system diseases. Many investigators consider that allodynia reflects central sensitization of second-order nociceptive neurons.[25] Animal studies showed that after nerve damage, owing to the ongoing spontaneous activity arising from primary nociceptors (peripheral sensitization), spontaneous activity in second-order nociceptive neurons increases, receptive fields enlarge, and responses to afferent impulses, including innocuous tactile stimuli, increase.[25] In this pathological condition, Aβ lowthreshold mechanoreceptors are able to activate second-order nociceptive neurons, thus gaining access to the pain-signalling pathway. Some previous studies in humans have provided support to the role of central sensitization and Aβ fibers for

the development of allodynia. Reaction time measurements demonstrated that allodynia is signaled by afferents with conduction velocities in the Aβ-fiber range.[26] Transcutaneous electrical stimulation of the skin area with allodynia evokes pain at stimulus intensities that produce nonpainful sensations in normal skin.[27] In chronic neuropathic pain, differential nerve blocks concurrently abolish allodynia and tactile sensation leaving Aδ and C-fiber-mediated modalities unaffected.[28] Evidence from humans has received support from many animal studies. After experimental nerve lesion, electrophysiological recordings from the dorsal horn documented that the second-order neurons exhibited high spontaneous discharges, enlarged receptive fields and lower thresholds, and augmented responses to mechanical stimulation.[29]

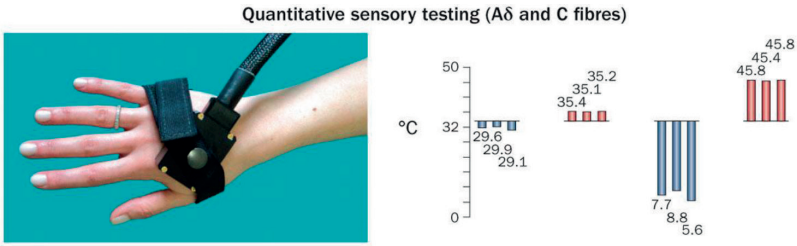
According to other investigators, rather than central sensitization, allodynia in patients with peripheral nervous system diseases might simply reflect peripheral sensitization.[30] Indirect support to this hypothesis comes from our studies using LEPs and skin biopsy (both techniques applied directly to the allodynic sites in patients with length-dependent neuropathy).[31]

Our LEP studies indicate that allodynia is associated with a relative sparing of nociceptive system. We strengthened this finding further in a skin biopsy study in patients with length-dependent neuropathy.[32] This study found a significantly higher epidermal nerve fiber density in patients with provoked pains (including dynamic mechanical allodynia) than in patients without these types of pain, thus showing that provoked pains and allodynia originate from relative nociceptive nerve fiber terminal sparing. These studies therefore indicate that in patients with length dependent neuropathy, a relative preservation of intraepidermal nerve fiber density and nociceptive system function increases the risk of allodynia. These findings are open to the interpretation that in several patients with painful neuropathy, dynamic mechanical allodynia and the other provoked pains might depend on a lowered mechanical threshold in hyperactive intraepidermal nociceptive nerve terminals. Accordingly, microneurographic studies show that light mechanical stimulation abnormally activates C nociceptors, thus producing allodynia.[33] All these findings in human studies indirectly raise the possibility that in some patients, peripheral sensitization might contribute, in addiction to second-order neuron sensitization to Aβ-fiber input, to the development of allodynia.

2. Methodologies used in the studies

2.1. Quantitative sensory testing

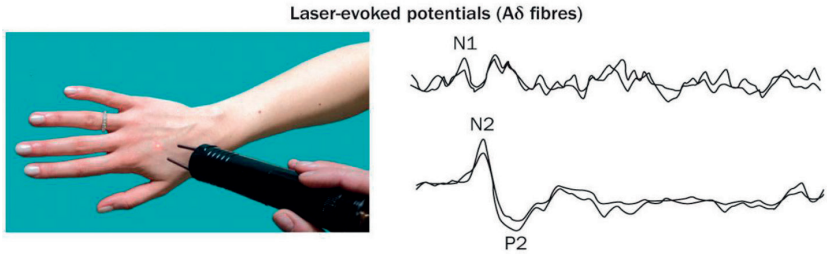
QST is a psychophysiological tool that measures perception of mechanical, thermal and painful stimuli delivered at controlled intensity. To determine the perceptive threshold for each sensation, stimuli are applied to the skin at increasing or decreasing intensities. Mechanical sensitivity for tactile stimuli is measured by producing graded pressures with plastic filaments (such as von Frey hairs), thermal perception and thermal pain are measured using a thermode or other devices that induce controlled temperature changes. QST can, therefore, assess function in nociceptive and non-nociceptive afferent pathways, and has proved to be a convenient tool for diagnosis and follow-up of small-fibre neuropathy that cannot be assessed with standard nerve conduction studies. [34]



2.2. Laser evoked potentials

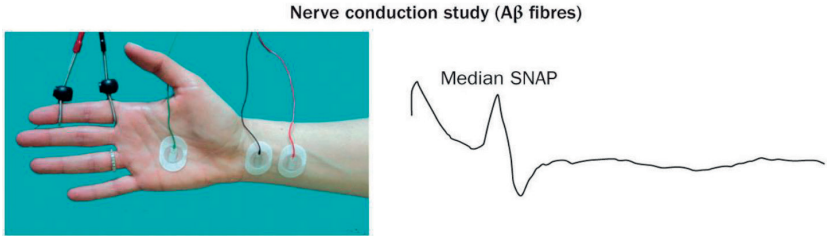
The easiest and most reliable neurophysiological technique for assessment of nociceptive pathway function is measurement of LEPs. For this technique, pulses of laser-generated radiant heat are used to selectively excite free nerve endings in the superficial skin layers, which activates Aδ and C nociceptors and gives rise to brain evoked potentials specifically related to activation of ascending thermal-pain systems.[35] The highest-amplitude scalp signal after a laser stimulus is a negative–positive complex maximal at the vertex. The highest-amplitude scalp signal after a laser stimulus is a negative–positive complex maximal at the vertex, resulting from the simultaneous activity of several cortical generators, with a major participation of the middle parts of the cingulate gyrus (MCC) and variable contribution from the insular and/or frontal operculum areas. An earlier, smaller negative wave (150–180 ms

after hand stimulation) is detected by the temporal leads, inverts polarity over the midline and is labelled "N1". EEG/MEG dipole analysis and intracortical recordings indicate that this signal is mainly generated in the upper bank of the sylvian fissure, encompassing the secondary somatosensory area (SII) and the posterior insula.[36]



2.3. Standard nerve conduction study

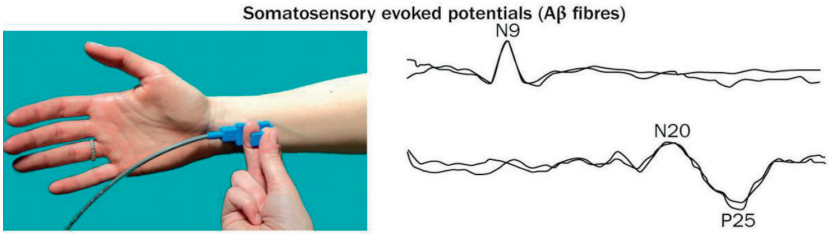
Nerve conduction study (NCS) is the standardized neurophysiological test used to evaluate the efficiency of electrical conduction of motor and sensory peripheral nerves, reflecting large myelinated Aβ fibres function. Sensory NCS is performed by electrical stimulation of a peripheral nerve and recording from a purely sensory portion of the nerve. The latency, i.e. the time it takes for the electrical impulse to travel from the stimulation to the recording site, is measured to calculate Nerve Conduction Velocity (NCV), whose slowing reflects Aβ fibres myelin loss. The amplitude of electrical response, named Sensory Action Potential (SAP) is also measured as a parameter whose reduction is primarily influenced by axonal impairment.[37]



2.4. Somatosensory evoked potentials (SEPs or SSEPs)

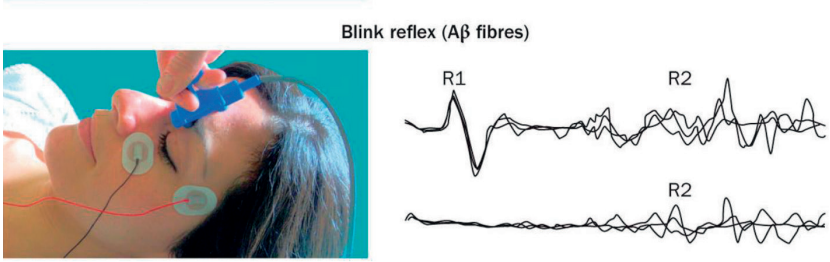
Somatosensory evoked potentials (SEPs or SSEPs) are a useful, non-invasive means of assessing somatosensory system functioning. By com-

bining SEP recordings at different levels of the somatosensory pathways, it is possible to assess the transmission of the afferent volley from the periphery up to the cortex. SEP components include a series of positive and negative deflections that can be elicited by virtually any sensory stimuli. For example, SEPs can be obtained in response to a brief mechanical impact on the fingertip or to air puffs. However, SEPs are most commonly elicited by bipolar trancutaneous electrical stimulation applied on the skin over the trajectory of peripheral nerves of the upper limb (e.g., the median nerve) or lower limb (e.g., the posterior tibial nerve) (i.e. Aβ fibers), and then recorded from the scalp.[38] In general, somatosensory stimuli evoke early cortical components (N20, P60, N80), generated in the contralateral primary somatosensory cortex (S1), related to the processing of the physical stimulus attributes. SSEP are a reliable technique to assess large non-nociceptive afferent fibres (Aβ fibre–dorsal column system), and are widely used for investigation of PNS and CNS diseases.



2.5. Blink reflex

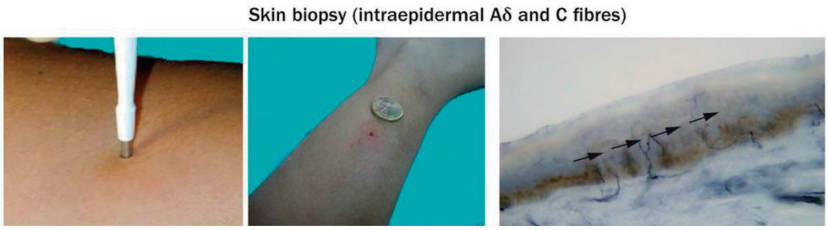
The orbicularis oculi reflex can be evoked by stimuli of various modalities. In clinical practice, a supraorbital nerve stimulus is often used to evaluate the trigemino-facial blink reflex. The blink reflex involves an early response (R1) ipsilateral to the stimulated supraorbital nerve and a late bilateral response (R2). The common afferent limb of the reflex loop is made up of the sensory trigeminal root and the ophthal-



mic division, whereas the common efferent limb consists of the facial nerve. It gives information about the integrity of large non-nociceptive afferent fibres (Aβ fibre).[39]

2.6. Skin biopsy

Skin biopsy is a reliable and minimally invasive tool for investigation of nociceptive fibres in human epidermis and dermis. Several techniques for assessment of the density of nociceptive skin nerve fibres exist, but normative ranges have only been standardized in the case of bright-field immunohistochemistry.The most commonly used markers for nerve fibres are antibodies against protein gene product 9.5 (PGP9.5), a ubiquitin carboxy-terminal hydrolase. PGP9.5 is widely distributed in the PNS and is a nonspecific panaxonal marker.[40]



3. Aim of the Ph.D project

The aim of this Ph.d project is to clarify mechanisms behind neuropathic pain with a particular focus on paroxysmal electric shock like sensation.

Neuropathic pain is very heterogeneous, with multiple patterns of presentation reflecting different combinations of etiological, genetic and environmental factors, and specifically, the neurobiological processes they engage. Because of their mechanistic diversity and different manifestations, these processes produce a complex profile or constellation of positive and negative sensory symptoms and signs, a ''*pain fingerprint*''.[41] To understand the genesis of the pain fingerprint we have to shift from etiologies to the reaction of the nervous system to the pathology. This is the cornerstone of the mechanistic approach to neuropathic pain. Moving from this assumption, during my Ph.D, I tried to extend the work by my group of research in help to disclose how mechanisms translate into symptoms. Our previous neurophysiological

studies in patients with neuropathic pain showed that while ongoing burning pain correlated with abnormalities of nociceptive fibre, paroxysmal electric-shock-like pain was associated with neurophysiological abnormalities involving non-nociceptive Aβ-fibres (Fig. 2) [8][10].

Thanks to these important findings we decided to apply a comprehensive neurophysiological approach to different types of neuropathic pain to disclose what set of fibers is mainly involved in generating a specific type of neuropathic pain. Our findings will surely be helpful in minimize pathophysiological heterogeneity within the groups under investigation and, thereby, be useful in drug trials and in tailoring therapy to the individual patient.

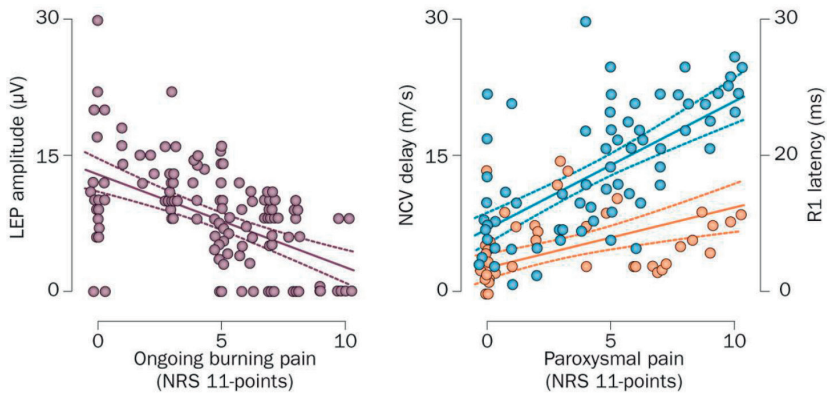


Fig. 2. | In a group of 150 patients with distal symmetric peripheral neuropathy or carpal tunnel syndrome, the intensity of spontaneous burning pain correlated with the LEP amplitude (Pearson r = 0.5015, P <0.0001). b | The intensity of paroxysmal pain correlated with the delay of median nerve sensory conduction velocity, calculated as 50 m/s minus the patient's NCV, in a group of 70 patients with carpal tunnel syndrome (blue circles; Pearson r = 0.6875, P <0.0001), and the R1-blink reflex latency in a group of 50 patients with postherpetic neuralgia (orange circles; Pearson r = 0.5278, P = 0.0001). Dashed lines indicate 95% CIs from the mean. Abbreviations: LEP, laser-evoked potential; NCV, nerve conduction velocity; NRS, Numerical Rating Scale.

Study I is a review that summarize our current understanding of the peripheral and central pathophysiological mechanisms underlying neuropathic pain and focus on how symptoms translate into mechanisms.

Study II is about the human distribution of C-units related to sensations of warmth, pain and

Itch and provides new information supporting the idea that specific unmyelinated C-units mediate sensations of warmth, burning and itch.

Study III is about the role of peripheral nociceptor sensitization in mediating allodynia in patients
with distal symmetric polyneuropathy.
Study IV is a neurophysiological study to demonstrate the role of large myelinated fibres in mediating paroxysmal pain.

3.1. Concluding Remarks

Neuropathic pain is such a huge theme to investigate and give an answer to the question "what's behind neuropathic pain?" it is an arduous challenge. I tried to approach the matter combining neurophysiological diagnostic tests and sensory profiles and I believe these data give a strong contribution to the new mechanisms-based approach. Furthermore the IV study definitively support the role of non-nociceptive Aβ-fibres in the development of spontaneous pain, such as the paroxysmal pain, thus breaking the dogma that only nociceptive fibres can mediate pain.

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Study I

Pathophysiological Mechanisms of Neuropathic Pain

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Introduction

The widely accepted definition of neuropathic pain is 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system'.[1]

Neuropathic pain is a frequent problem in many peripheral and CNS diseases. The peripheral nerve diseases that most commonly cause neuropathic pain are distal symmetric peripheral neuropathies (e.g., diabetic neuropathy) and focal neuropathies related to trauma (e.g., traumatic brachial plexus injuries), as well as surgical interventions (e.g., breast surgery). Exemplary CNS diseases causing neuropathic pain include multiple sclerosis, spinal cord injury and stroke. The widely ranging etiologies suggest a high prevalence of neuropathic pain in the general population. Postal surveys designed to investigate chronic pain with neuropathic characteristics in large community samples have reported a 7–8% prevalence of neuropathic pain in the general population.[2,3]

Neuropathic pain arises through multiple and complex pathophysiological mechanisms. Convincing evidence on the relationship between the underlying pathophysiological mechanisms and neuropathic pain symptoms now suggests that classifying neuropathic pain according to a mechanism-based rather than an etiology-based approach might help in targeting therapy to the individual patient and would also be useful in testing new drugs. In this article we summarize our current understanding of the peripheral and central pathophysiological mechanisms underlying neuropathic pain and focus on how symptoms translate into mechanisms.

Mechanisms Underlying Neuropathic Pain

Animal Models

Most of our current knowledge on the complex pathophysiological processes that trigger neuropathic pain comes from animal models of peripheral nerve injuries, largely designed to mimic human diseases.[4] Although these models have the important merit of improving our knowledge on the mechanisms underlying neuropathic pain, they often poorly predict the involvement of particular targets or processes in human neuropathic pain.[4] Several studies have used total nerve transection and ligation to simulate the clinical conditions of amputation.[5] Partial nerve ligation[6] and spared nerve injury[7] have been used to simulate the clinical condition involving partial peripheral nerve injury. Spinal nerve ligation effectively simulates spinal root damage owing to a lumbar disk herniation.[8] Immune or toxin-mediated demyelination simulates demyelinating neuropathy.[9] Vincristine, paclitaxel and cisplatin have been used in animal models to mimic polyneuropathy caused by tumor chemotherapy.[10] Finally, streptozocin-induced damage to pancreatic insulin-producing cells in rats provides an experimental model of diabetic neuropathy.[11]

Peripheral Sensitization

Following nerve damage, a neuroma, consisting of regenerative nerve sprouts growing in all directions, develops at the proximal nerve stump. Electrophysiological recordings demonstrate that after nerve damage, ongoing spontaneous activity, abnormal excitability and an increased sensitivity to chemical, thermal and mechanical stimuli develop at multiple sites, including the neuroma (the site of injury with aborted axon growth), in the cell body of injured dorsal root ganglia neurons[12] and in neighboring intact afferents.[13] This 'hyperactivity' involving the nociceptive primary afferents is defined as peripheral sensitization (Fig. 1).[4,14–18]

Peripheral sensitization arises through various pathophysiological mechanisms. Following nerve damage, voltage-gated sodium channel expression undergoes marked changes. Many studies demonstrated abnormal sodium channel Nav1.3, Nav1.7, Nav1.8 and Nav1.9 expres-

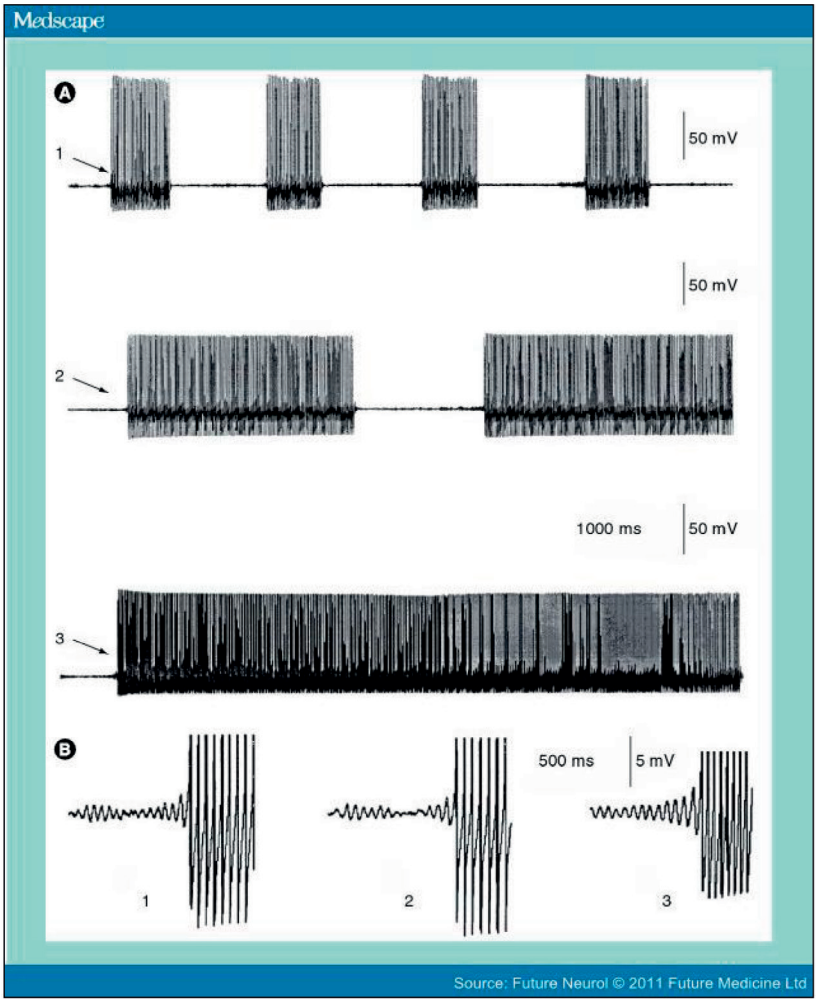

Fig. 1. A high-frequency discharge recorded in primary afferents after spinal nerve injury. In all of the trains, the spikes **(A, 1–3)** are always triggered by a subthreshold oscillation peak as seen in the expanded time and voltage scale **(B, 1–3)**. Reproduced with permission from [123].

sion,[19,20] leading to primary afferent hyperexcitability (a lowered threshold and higher firing rate).[14,21,22] Clusters of sodium channels accumulate at the site of the nerve lesion but also within the intact dorsal root ganglion. In the dorsal root ganglion there is a phasically activating, voltage-dependent sodium conductance alternating with a passive, voltage- independent potassium leak, generating characteristic membrane potential oscillations. When oscillation sinusoids reach threshold amplitude, ectopic firing ensues (Fig. 2).[23,24]

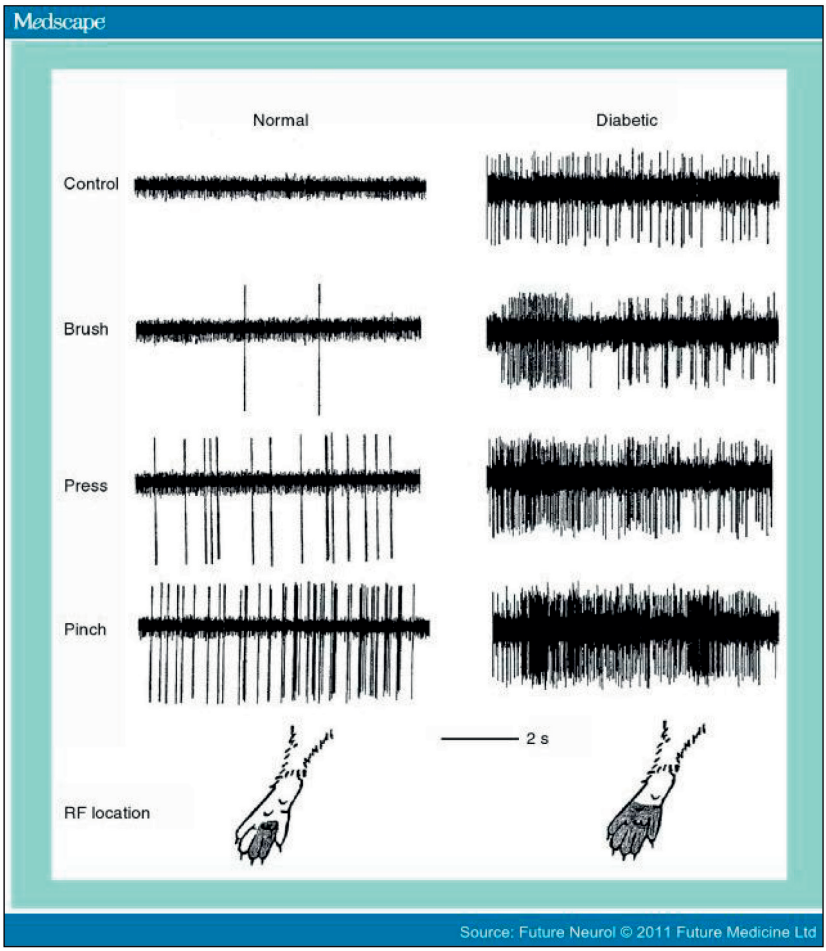

Fig. 2. Baseline activity and responses to brush, press and pinch in one normal and one diabetic wide dynamic range neuron. The RFs of these two spinothalamic tract neurons are indicated in the shaded area of the rat hindpaw.
RF: Receptive field.
Reproduced with permission from [124].

A useful animal model of neuropathic pain that involves dysregulated sodium channel expression in dorsal root gangli Ab-fibers (see for the glossary) on neurons is streptozotocin-induced diabetes. In this model sodium-channels Nav1.3, Nav1.6 and Nav1.9 mRNA and protein expression is upregulated, and *Nav1.8* mRNA is downregulated.[21,25,26] Whole-cell patch-clamp recordings demonstrated an increase in the peak current density and ramp current amplitude, consistent with Nav1.3, Nav1.6 and Nav1.7 channel upregulation, which

produces robust ramp currents.[27] The type III embryonic sodium channel (Nav1.3) probably plays a key role in the development of neuropathic pain. It is present at low levels in adult afferent nociceptive pathways and after an experimental nerve injury its expression markedly increases.[28–30] It rapidly recovers from inactivation and has slow closed- state inactivation kinetics, suggesting that neurons expressing Nav1.3 may exhibit changes in either reduced threshold or a relatively high firing frequency, orboth.[28–30]

Box 1. Glossary.

- Aβ-fibers: large-myelinated nerve afferents or pathways that convey non-nociceptive input (e.g., tactile sensation)
- Aδ-fibers: small-myelinated nerve afferents or pathways that convey cold and nociceptive input
- Allodynia: pain sensation induced by a stimulus that normally does not provoke pain, and thus implies a change in the quality of a sensation
- Blink reflex: neurophysiological tool for assessing trigeminal large-myelinated pathway
- C-fibers: unmyelinated nerve afferents or pathways that convey thermal and nociceptive input
- Catechol- *O* -methyltransferase: enzyme that degrades catecholamines such as dopamine, epinephrine and norepinephrine
- Central sensitization: increased background activity, enlarged receptive field and increased responses to all afferent impulses of the second order nociceptive neurons
- Dysesthesias: spontaneous, nonconstant sensations that are clearly unpleasant (e.g., pins and needles)
- Hyperalgesia: increased pain response to a stimulus that normally provokes pain (e.g., the pin used in neurological examination)
- Laser-evoked potentials: scalp signals evoked by laser stimuli, which selectively assess Aδ afferent pathways
- Nerve Conduction Study: the standard electrodiagnostic tool for assessing peripheral nerve fiber function. It assesses only Aβ-fibers
- Paresthesia: spontaneous, nonconstant sensations that are not clearly unpleasant(e.g., tingling)

- Paroxysmal pain: sudden, very short-lasting pains (e.g., electric-shock-like, stabbing sensations)
- Peripheral sensitization: a reduction in threshold and an increase in responsiveness of the peripheral ends of nociceptors.
- Skin biopsy: a minimally invasive technique that assesses the density of intraepidermal fibers, which mainly consist of C-fibers
- TRPV1: the transient receptor potential cation channel, subfamily V, member 1. Also known as the capsaicin receptor, TRPV1 is a nonselective cation channel expressed predominantly in unmyelinated C-fibers
- Wide dynamic range neurons: second-order neurons located in the spinal cord dorsal horn, responsive to all sensory modalities (thermal, chemical and mechanical) and a broad range of intensity of stimulation from primary afferentsm

These data, together with experimental and clinical observations on the partial effectiveness of sodium-channel blocking agents in neuropathic pain, established a link between sodium channel activity and primary afferent hyperexcitability producing pain.

[31] Recent studies have linked gain-of-function mutations in *SCN9A*, the gene that encodes Nav1.7, to two human-inherited pain syndromes, inherited erythromelalgia and paroxysmal extreme pain disorder, whereas loss-of-function mutations in *SCN9A* have been linked to complete insensitivity to pain.[32,33]

Although potassium channel expression has been studied less than sodium channel expression in animal models of neuropathic pain, potassium-channels probably have a key role in the development of neuropathic pain. Several studies reported a reduction in potassium channel transcript expression in the dorsal root ganglion after peripheral nerve lesions.[34–36] Furthermore, potassium channel openers act as analgesics in animal models of neuropathic pain.[37,38]

The development and maintenance of peripheral sensitization is modulated by cytokines, small proteins involved in inflammatory processes. Various animal experiments demonstrate that peripheral nerve injury increases TNF and IL-1β immunoreactivity in dorsal root ganglia of both injured and uninjured ipsilateral adjacent afferents.[39] The increased cytokine level is associated with reduced mechanical and thermal

withdrawal thresholds in rats.[40–44] Epineurially-applied TNF elicited acute mechanical hyperalgesia in the awake rat[40] and antibodies neutralizing the TNF receptor injected at the site of nerve injury reduce pain behavior in mice.[41] Exogenous TNF injected into dorsal root ganglia of damaged roots is transported into the dorsal horn, precipitating allodynia in both the ligated and adjacent uninjured nerves.[42,43] Nerve biopsies of patients with painful neuropathies demonstrated higher TNF immunoreactivities in myelinating Schwann cells and serum solubleTNF receptor levels are higher in patients with centrally-mediated mechanical allodynia.[44] An endogenous IL-1β receptor antagonist, experimentally injected in mice, prevents inflammatory hyperalgesia, and antibodies neutralizing IL-1β receptors reduce pain-associated behavior in mice with experimental nerve damage.[45–47] After unilateral chronic constriction injury, IL- 1β also increases in the contralateral homolog nerve.[48,49] This selective contralateral cytokine induction is probably mediated by NMDA receptors and reflects a spinal mechanism.

Peripheral sensitization also involves the upregulation of various proteins, some of them only marginally expressed under physiological conditions. Various animal studies demonstrated that peripheral nerve injury changes transient receptor potential (TRP) channel expression. TRP channels are a family of non-selective cation-permeable channels that are known to be important for sensory signaling in the peripheral nervous system. Several animal studies investigated the role of the vanilloid receptor 1 (TRPV1), a member of the TRP family, in the development of neuropathic pain.[50–53] Total or partial sciatic nerve transection, or spinal nerve ligation, reduce TRPV1 expression in the somata of all damaged dorsal root ganglia.[50–53] Following partial nerve lesion or spinal nerve ligation, TRPV1 expression is greater in the undamaged dorsal root ganglion somata than in controls.[50–53] Evidence that hyperalgesia does not develop in TRPV1-deficient mice and that TRPV1 antagonists reduce pain behavior in mice after spinal nerve ligation further supports the idea that TRPV1 plays a crucial role in the development of neuropathic pain.[50–53]

Normal nerve terminals assume signal substances that are transmitted by axonal transport to the dorsal root ganglion cell body. In the dorsal root ganglion cell body these signal substances modify gene transcription and protein synthesis.[54,55] After nerve damage, sprouts can no longer assume these molecules. Therefore, nerve damage, through complex signaling mechanisms (cAMP-dependent PKA

and Ca2+/phospholipid-dependent PKC) modulate gene transcription. Animal studies demonstrated that after nerve damage, there is an induction of *c-jun*, *p-38* and *ERK*.[56–60] The encoded proteins of these genes are involved in inflammatory responses, neuronal degeneration and neuronal plasticity, which maintain pain sensation. [14,56–60] Therefore, the importance of genetic factors in neuropathic pain remains an interesting question for further research, especially for their possible use as targets for new, more selective drugs.

In accordance with findings from animal studies, microelectrode recordings from transected nerves in human amputees with phantom limb pain, displayed spontaneous afferent activity (i.e., peripheral sensitization). In these patients, tapping the neuroma increases pain and afferent discharges.[61] The injection of lidocaine into the neuroma blocks nerve activity owing to the tap of the neuroma and its related pain.[16] By contrast, perineuromal injection of gallamine, a potassium channel blocker, increases pain.[62] Some investigators demonstrated an inverse relationship between ongoing pain and heat pain deficit in patients with postherpetic neuralgia.[63] In these patients, lidocaine applied to the painful skin in patients with postherpetic neuralgia produces effective pain relief.[64] Microneurographic studies demonstrated that in patients with peripheral neuropathies, pain is associated with ongoing spontaneous firing of unmyelinated c-fibers.[65–67]

Central Sensitization

Despite the increasing evidence underlying the importance of peripheral sensitization, many investigators consider central sensitization the main pathophysiological mechanism responsible for neuropathic pain.[14,68–70] The primary afferent pathways that convey human pain signals connect in the spinal cord dorsal horn with second-order nociceptive neurons. They consist of nociceptive-specific neurons and wide dynamic range neurons.[71,72] Nociceptive-specific neurons are located in the outer layers (laminae I–II) of the dorsal horn; wide dynamic range neurons lie in deeper laminae (most of lamina V neurons are wide dynamic neurons). Nociceptive-specific neurons respond selectively to noxious stimuli conveyed by Aδ- and C-fibers. Wide dynamic range neurons excited both by noxious and non-noxious stimuli, receive both large-myelinated Aβ-fibers as well as Aδ- and C-fibers. Wide dynamic range neurons can encode and project different types

of sensory information, nociceptive and non-nociceptive, varying their firing rate (higher for noxious and lower for non-noxious stimuli). Nociceptive neurons have a fairly localized receptive field and probably play an important role in spatially detecting nociceptive stimuli. By contrast, since wide dynamic range neurons have a large receptive field and a stimulus-response function (the higher the stimulus intensity, the higher the firing rate of their output), their main function is to detect and discriminate the intensity ofnoxious stimuli.[73,74]

Animal studies demonstrated that after nerve damage, owing to the ongoing spontaneous activity arising from primary nociceptors (peripheral sensitization), background activity in second-order nociceptive neurons increases, receptive fields enlarge and responses to afferent impulses, including innocuous tactile stimuli, increase (Fig. 2). In this pathological condition, Aβ low-threshold mechanoreceptors can activate second-order nociceptive neurons, thus gaining access to the pain- signaling pathway. This phenomenon is termed central sensitization.[4,16,68,75] Central sensitization has been documented in animals and may explain persistent neuropathic pain in patients.[14,69,70]

Peripheral nociceptor hyperactivity causes major secondary changes in the spinal cord dorsal horn. In response to pain stimuli, the central terminals of primary nociceptive afferents in the dorsal horn of the spinal cord release the neurotransmitters glutamate and substance P, as well as brain-derived neurotrophic factor. The amino acid glutamate, the major excitatory neurotransmitter found throughout the whole nervous system, is essential for pain signaling at every anatomical level. Primary nociceptive afferents release glutamate in response to acute and persistent noxious stimuli, and through AMP acid(AMPA) receptor activation, set the initial baseline response of spinal dorsal horn neurons. Delivering repetitive and high-frequency stimulation to primary nociceptive afferents amplifies and prolongs the responses of spinal dorsal horn neurons. This enhanced activity results from NMDA-receptor activation. Acute or low-frequency stimuli delivered to second-order neurons cannot activate the NMDA receptor because in normal physiological conditions the magnesium ion (Mg2+) levels found in nervous tissues block the receptor's ion channel. A sustained membrane depolarization is required to activate and open the NMDAreceptor-channel.

[72] The contact between neurotransmitters and receptors produce an increase of intracellular Ca2+ and cAMPconcentrations, which acti-

vates protein kinases. Protein kinases consist of the signaling cascade that modulates gene transcription (i.e., *c-fos*, *c-jun*).[69,76]

Like peripheral sensitization in neuropathic pain, recent studies demonstrate that central sensitization arises also through changes in ion channels. Peripheral nerve injury leads to changes in sodium-channel expression within nociceptive dorsal horn neurons, strongly suggesting that sodium channel changes in the dorsal horn contribute to neuropathic pain. For example, experimental spinal cord injury upregulates Nav1.3 in dorsal horn neurons. This upregulation is associated with hyperexcitability in second-order nociceptive neurons and pain. Antisense knockdown of Nav1.3 reduces second-order nociceptive neuronal hyperexcitability and pain behavior in spinal cord-injured rats.[77,78] Several lines of evidence suggest that the mechanisms underlying central sensitization at the dorsal horn level also involve molecular mechanisms other than sodium channels, for example, prostaglandins and cytokines, the proinflammatory substances that facilitate pain transmission.[14,22,79]

Although most investigators consider central and peripheral sensitization as the main mechanisms underlying neuropathic pain, peripheral nerve damage also leads to other central changes.[79] For example, mild afferent signal loss might induce major changes in dorsal horn neuron excitability. When large Aβ-fiber input decreases, the interneurons that inhibit nociceptive neurons become hypoactive (loss of afferent inhibition).[80] Earlier research suggested changes in the descending modulatory systems[81] subsequently confirmed by the efficacy of serotonin and noradrenalin reuptake-blocking antidepressants in neuropathic pain.[14] During massive deafferentation, after presynaptic terminal buttons are lost, the postsynaptic receptors on spinothalamic tract (STT) neurons become exposed to neurotransmitters, and STT neurons begin to fire spontaneously (deafferentation supersensitivity).[14]

This article again underlines the role of glial cells in neuropathic pain. Glial cells, including microglia and astrocytes, are non- neuronal cells that have various functions in the spinal cord. Glial cells act as physical support, release mediators that modulate neuronal activity and alter axonal and dendritic growth. Under normal conditions they account for 70%of CNS cells. [82]

Several lines of evidence indicate that spinal cord microglia and astrocytes are implicated in creating exaggerated pain states.[83–87] Glial cells play a crucial role in maintaining neuronal homeostasis in the CNS and immune factors produced by microglia are believed to play

an important role in nociceptive transmission. Increasing evidence demonstrates that uncontrolled glial cell activation under neuropathic pain conditions induces the release of proinflammatory cytokines and other substances that facilitate pain transmission.[83–87] Glial cells also enhance the release of substance P and excitatory amino acids from nerve terminals, including primary afferents in the spinal cord.[83,84] Glial cell activation can also lead to altered opioid system activity.[85–87] During strong neuronal excitation, such as that induced by neuropathic pain, fractalkine, a protein expressed by neurons, breaks free.[88] The soluble portion of fractalkine diffuses away and binds to and activates glial cells.[89] Intrathecal fractalkine creates both thermal hyperalgesia and mechanical allodynia, and fractalkine receptor blockade blocks inflammatory neuropathy-induced pain.[90]

Mechanism-based Symptoms

At the bedside examination, neuropathic pain can been distinguished from spontaneous pain, (i.e., stimulus independent) and provoked pain.[70] Spontaneous pain can have several different qualities. The most typical spontaneous pains are ongoing pain (usually superficial burning or deep pressing pain, or both), and paroxysmal pain (electrical shock-like, stabbing pain).[79,91] Provoked pain includes allodynia, pain in response to a normally nonpainful stimulus, and hyperalgesia, an increased response to a normally painful stimulus. Unfortunately, unlike animal studies, neuropathic pain mechanisms in humans remain largely unclear; current clinical and neurophysiological research has proposed various mechanismsfor each type of pain.

A useful way to draw parallels between symptom and mechanism is to combine patients' sensory profiles, obtained by specific questionnaires such as the Neuropathic Pain Symptom Inventory (NPSI), using data obtained with neurophysiological tools (blink reflex, nerve conduction studies and laser-evoked potentials).

Patients with neuropathic pain syndromes typically describe their pain as constant and burning. In a group of 150 patients with various types of polyneuropathy (68 with neuropathic pain) approximately 90% complained of burning pain.[92] Previous neurophysiological studies demonstrated that in patients with various neuropathic pain conditions (postherpetic neuralgia, carpal tunnel syndrome

and polyneuropathy) burning pain is associated with nociceptive pathway damage as assessed by laser-evoked potential recordings (Fig. 3).[92–94] Microneurographic studies demonstrated that in patients with peripheral neuropathies the spontaneous burning pain was associated with the ongoing spontaneous firing of C fibers.[65–67] Skin biopsy studies described reduced intraepidermal nociceptive terminals in patients with ongoing pain related to peripheral neuropathy. [95,96] These data suggest that ongoing burning pain is probably due to the abnormal spontaneous activity originating in damaged nociceptive fiber axons that have lost their intraepidermal endings. Although the spontaneous activity causing burning pain presumably originates from axonal sprouts, a concurrent mechanism might include long-term CNS changes provoked by nociceptive pathway damage, such as hyperactivity in the second-order neurons (central sensitization).[22,97] A recent microneurographic study provided new evidence of a specific C-fiber set that have a bimodal thermoreceptive properties and are activated by cooling, heating and menthol.[98] Activity of this specific set of C-fiberscould be responsible for the stinging, hot and burning sensations evoked by innocuous cold stimuli.[99] Ongoing burning pain might also be related to the central hyperactivity resulting from deafferentation. In patients with postherpetic neuralgia, the ongoing burning pain is associated with a severe heat pain deficit, thus suggesting a severe C-afferent-fiber loss. A previous study used the C-fiber-mediated histamine axon reflex in patients with postherpetic neuralgia to determine C-fiber activity, demonstrating an abolished response in the area of maximum pain.[16] Ongoing burning pain frequently manifest as sequelae related to deafferentation, produced by a brachial plexus avulsion. Direct recordings of spinal neuron activity in a patient with injury to the dorsal roots of the cauda equina disclosed high-frequency, regular and paroxysmal bursting discharges.[16] The patient suffered from spontaneous burning pain in a region where the lesion had caused anesthesia (anaesthesia dolorosa).

Previous neurophysiological studies in patients with postherpetic neuralgia and carpal tunnel syndrome demonstrated that paroxysmal pain is associated with abnormalities involving non-nociceptive Aβ-fibers.[93,94] More specifically, in patients with postherpetic neuralgia and carpal tunnel syndrome, the correlation between the blink reflex delay and median-nerve sensory conduction velocity slowing, sug-

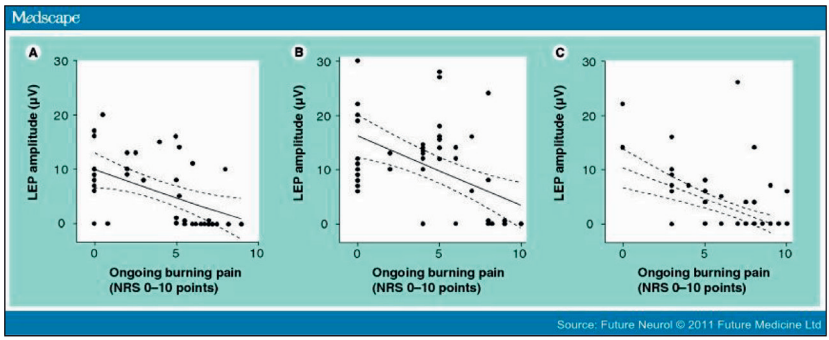

Fig. 3. Correlations between the severity of ongoing burning pain and laser-evoked potential abnormalities in various neuropathic pain conditions. **(A)** 41 patients with ophthalmic postherpetic neuralgia. LEPs elicited from supraorbital stimulation. **(B)** 40 patients (75 hands) with carpal tunnel syndrome. LEPs elicited from the hand (median nerve territory). **(C)** 150 patients with polyneuropathy. LEPs elicited from the foot. The more severe the burning pain, the more abnormal the LEPs, changes that reflect nociceptive pathwaydamage.
LEP: Laser-evoked potential.

gests that this type of pain is related to focal Aβ-fiber demyelination. In accordance with previous studies in animals describing spontaneous ectopic discharges recorded in large myelinated Aβ-fiber axons after nerve injuries,[9,100,101] paroxysmal pain may be related to high-frequency bursts generated in demyelinated Aβ-fibers. It is still unclear whether these high-frequency bursts in demyelinated Aβ-fibers are sufficient to provoke pain *per se* or do so only after ephaptic transmission to the neighbouring unmyelinated C-fibers, or by involving wide dynamic range neurons.[94] Although most investigators consider paroxysms as peripheral phenomena related to spontaneous firing, a clinical study provided evidence that paroxysmal pain is associated with decreased small-fiber function, thus raising the possibility that paroxysms originate centrally in the second-orderneurons.[102]

No general agreement exists regarding the pathophysiological mechanism underlying allodynia.[18] Two opposing views currently exist, one peripheral[67,103] and the other central.[104] According to some investigators, allodynia reflects peripheral sensitization.[105] Over the past decades, a possible role for hyperexcitable peripheral nociceptors as primary determinants of pain in humans has received ample support. Microneurographic recordings in patients with painful neuropathy demonstrated that allodynia was related to C nociceptor firing.[67] A recent study in patients with polyneuropathy found that allodynia was associated with a relative sparing of nociceptive fibers,

as assessed with laser-evoked potentials.[92] These findings suggest that allodynia reflects an abnormal reduction in the mechanical threshold in sensitized peripheral nociceptors.[79,92,106]

According to many investigators, allodynia is generated at a central level.[16,18] The spontaneous firing in damaged nociceptive afferents may evoke ongoing pain and, as a secondary effect, sensitize central nociceptive neurons.[68,107–109] As a result, a large skin area surrounding the initial lesion site may become hypersensitive to light touch (i.e., allodynia). Microneurographic studies demonstrated that allodynia is mediated by large myelinated Aβ-fiber low-threshold mechanoreceptors.[110] In chronic neuropathic pain, differential nerve blocks demonstrate that allodynia is abolished concomitantly with loss of innocuous tactile sensation at a time when Aδ- and C-fiber mediated modalities are unaffected.[107,111] In patients with neuropathic pain, a selective Aβ-fiber block eliminates allodynia[107,112] but ongoing burning pain persists, indicating that it is mediated by C- nociceptors.[16] Central sensitization as the main mechanism underlying allodynia also receives support from the link between this pain symptom and abnormal pain summation on repetitive mechanical stimulation, a sign of central sensitization.[16]Future research efforts, designed to translate mechanisms into symptoms, should therefore seek more information to clarify the peripheral mechanisms underlying neuropathic pain.

Sensory Profiles

Patients experiencing neuropathic pain suffer from sensory deficits, as well as various types and different combinations of pain. Neuropathic pain may be ongoing (e.g., burning and pressing), paroxysmal pain (e.g., stabbing and electric shock-like sensations) or pain provoked by various stimuli (e.g., gentle brushing [allodynia] or cold water [cold allodynia]). Specific types of pain may predominate in some neuropathic pain conditions but none of them are etiologic specific.[113,114] Thus, patients suffering from the same disease may present with a heterogeneous profile of symptoms and sensory signs. Therefore, the aim of diagnostic workup should be to define specific sensory profiles through clinical examination, questionnaires dedicated to neuropathic pain and laboratory tools.

Current research findings strongly indicate that the different profile of sensory signs and symptoms, (including provoked pain and spon-

taneous pain) arise through different pathophysiological mechanisms. Clinical, neurophysiological and neuropathological investigations show that in patients with peripheral neuropathy of various etiologies, spontaneous burning pain is invariably related to the nociceptive pathway damage.[92–95] By contrast, recent neurophysiological studies suggest that spontaneous paroxysmal pain reflects demyelination of non-nociceptive, large-myelinated fibers (as described previously).[93,94] Overall, these findings suggest that neuropathic pain can be classified by sensory profiles (quality of pain) rather than etiology, as the recent European guidelines recommend.[115] Classifying neuropathic pain according to a mechanism-based rather than an etiology-based approach might minimize pathophysiological heterogeneity within the groups under study and thus help in targeting therapy to the individual patient.

Genetic Inheritance of Neuropathic Pain

Because not all patients with nerve injury experience neuropathic pain, the heritable predisposition for neuropathic pain probably varies between subjects. Animal studies indicate that neuropathic pain sensitivity encompasses a large heritable component,[116] hence genetic risk factors are probably important in the various clinical neuropathic pain conditions.[117]

Some genetic diseases are associated with an increased risk for the development of neuropathic pain. For example, Fabry disease is a rare X-linked recessive (inherited) lysosomal storage disease that causes painful neuropathy.[118] Gain-of-function mutations in *SCN9A*, the gene that encodes Nav1.7, cause two extremely rare inherited neuropathic pain conditions, erythromelalgia and paroxysmal extreme pain disorder.[32] In these rare conditions traditional genetic techniques can be applied for studying genetic susceptibility. Yet, because the nervous system diseases that most commonly cause neuropathic pain are sporadic, neither family history nor classic genetic techniques can be relied upon to evaluate the heritable susceptibility to this condition. Reasonably, the genetic risk of developing neuropathic pain after nervous system damage results from multiple risk-conferring genes. In an attempt to highlight the role of genetic susceptibility in neuropathic pain, Costigan and colleagues (2010) investigated a single nucleotide polymorphism association of the potassium channel α subunit, KCNS1, in humans with

neuropathic pain.[117] They found that a common amino acid changing-allele, the 'valine risk allele', was significantly associated with higher pain scores. Other studies investigated catechol-O-methyltransferase polymorphisms that modulate nociceptive and dysfunctional temporomandibular joint disorder pain.[119,120] A recent study demonstrated that a single nucleotide polymorphism in *SCN9A* increased firing frequency of DRG neurons; this single nucleotide polymorphism was subsequently shown to be associated with chronic pain.[121,122] Therefore, in defining sensory profiles we need to take into account the increasing evidence that each patient has a unique genomic fingerprint. A new future approach to neuropathic pain should therefore include genetic analysis among the more conventional diagnostic tools.

Conclusion

Neuropathic pain arises directly from a lesion or disease affecting the somatosensory system. Our current knowledge on the mechanisms of neuropathic pain comes largely from animal models of peripheral nerve injury. Animal models demonstrate that after a peripheral nerve injury, spontaneous activity develops in damaged axons, excitability becomes abnormal and sensitivity to chemical, thermal and mechanical stimuli increases, resulting in the development of peripheral sensitization. Owing to the ongoing activity arising from primary afferents, background activity in second-order nociceptive neurons increases, receptive fields enlarge and responses to all afferent impulses increase, resulting in the development of central sensitization.

Although animal models help to understand the mechanisms responsible for neuropathic pain, they poorly reflect clinical conditions. Therefore, data from animals cannot invariably be applied in humans. In humans, different pathophysiological mechanisms are responsible for the development of neuropathic pain that manifests with heterogeneous sensory disturbances. Although specific types of pain may predominate in some etiological categories of neuropathic pain, none of them are etiology- specific. Thus, regardless of the disease, patients suffering may present with heterogeneous sensory signs and symptoms, even with the same disease. Our findings in this article show that we now have the information needed for classifying neuropathic pain according to a mechanism-based, rather than an etiology-based, approach and targeting therapy to the individual patient.

Future Perspective

Neuropathic pain owing to lesions or disease of the nervous system remains a major neurological challenge. We now have to change the way we classify, diagnose and treat neuropathic pain from an etiology-based to a mechanism-based approach. In clinical practice, the diagnostic work-up should aim at defining specific sensory profiles, thus targeting therapy to the individual patient and improving drug testing. Although a possible future direction for managing neuropathic pain might be mechanism- based therapy, clinical experimental studies indicate that a specific symptom might be generated by several entirely different underlying pathophysiological mechanisms, suggesting a wider phenotypical approach to the patient with neuropathic pain.

Future studies should also clarify how genetic factors contribute to the risk of neuropathic pain. Improved knowledge of the genes involved in neuropathic pain conditions might help us in targeting novel analgesics and biomarkers of neuropathic pain.

Executive Summary

Mechanisms Underlying Neuropathic Pain

- Electrophysiological recordings demonstrate that the regenerating C-fibers of damaged axons develop ongoing spontaneous activity, abnormal excitability and an increased sensitivity to chemical, thermal and mechanical stimuli. This phenomenon is termed peripheral sensitization.
- Following nerve damage, as a consequence of the peripheral sensitization, second order nociceptive neurons develop an increased background activity, enlarged receptive field and increased responses to all afferent impulses. This phenomenon is termed central sensitization.

Mechanism-based Symptoms

- Neuropathic pain may be ongoing (e.g., burning pain), paroxysmal (e.g., electrical shock-like sensations) or provoked by various stimuli. The different types of neuropathic pain probably arise through variations in the underlying mechanisms.

- Burning pain probably reflects the abnormal, spontaneous activity originating in damaged nociceptive fiber axons.
- Paroxysmal pain may be related to high-frequency bursts generated in demyelinated Aβ-fibers.
- Allodynia may be due to a peripheral mechanism, reflecting an abnormal reduction of the mechanical threshold in sensitised nociceptors, or to a central mechanism, reflecting the sensitization of central nociceptive neurons to mechanically evoked input.
- Successful neuropathic pain management requires the definition of precise sensory profiles. The diagnostic process should aim at finding specific sensory profiles through clinical examination, questionnaires dedicated to neuropathic pain and laboratorytools.
- A classification per sensory profile rather than etiology might minimize pathophysiological heterogeneity and increase the power to detect a positive treatment result.

Genetic Inheritance of Neuropathic Pain

- Reasonably, the genetic risk of developing neuropathic pain after nervous system damage results from multiple risk- conferring genes.

Future Perspective

- An increased knowledge of the mechanisms underlying pain and their translation into signs and symptoms in patients might lead to an optimal therapeutic approach, with drugs that address the specific combination of mechanisms occurring in each patient.

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Study II

Topographical distribution of warmth, burning and itch sensations in healthy humans

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Introduction

Unmyelinated C-fibre units mediate widely ranging sensations in humans, and are usually distinguished according to their response to mechanical, heat and chemical stimuli. C-units responding to mechanical and heat stimuli include mechano-heat-sensitive (CMH) or C-polymodal nociceptors [6,24]; other C-unit receptors are insensitive to mechanical stimuli though they may respond to heat. Whereas C-polymodal nociceptors and most C-units insensitive to mechanical stimuli respond to capsaicin application, mechano-insensitive C-units alone respond to histamine application [7,20,22].

In humans, laser stimuli activate Aδ- and C-fibre related receptors, evoking brain responses and a variety of sensations [18,27]. Large-spot and low-intensity laser pulses selectively activate warmth C receptors because they have a markedly lower thermal activation threshold and density than the other C-fibres related receptors [5,28]. Capsaicin strongly activates polymodal nociceptors through vanilloid-gated ion channels [3], predominantly producing burning sensations. Histamine directly activates mechano-insensitive C-units and is widely used to induce experimental itch [9,10].

The currently preferred techniques for investigating C-unit properties in humans are microneurography and skin biopsy [17,23,25]. Although microneurographic studies can accurately identify the various C-unit subclasses they provide no information on their body distribution. Similarly, although skin biopsy studies detect a higher density of epidermal free nerve endings in proximal than in distal leg sites they can neither distinguish between Aδ- and C-units, nor be-

tween the various C-unit subpopulations. No information is available on how the various C-units are distributed in the various body sites, a key point in understanding pain and itch arising from unmyelinated pathway damage.

In this psychophysical study, to seek information on the body distribution of C-units related to sensations of warmth, pain and itch we delivered laser stimuli, applied capsaicin cream and pricked histamine onto the skin in hairy body sites at various distances from the brain in healthy subjects and investigated differences in the following outcome variables: magnitude of sensations, diameter of the flare, and secondary hyperalgesia and alloknesis (the itchy area surrounding the wheal and flare).

Material and methods

Twelve healthy subjects (age 25–35 years) who gave their informed consent, participated in the study. None of them had known allergies. All the subjects underwent the application of all the three stimuli in each body site tested. This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board of the Department of Neurological Sciences, University Sapienza.

To investigate warmth sensation we used a neodymium:yttrium–aluminium–perovskite laser (Nd:YAP by El.En., Florence): wavelength 1.34 nm, pulse duration 2–20 ms, maximum energy 7 J, under fiberoptic guidance. Laser pulses of lower intensity (38–76 mJ/mm2), relatively long duration (10 ms) and large diameter (10 mm), elicited a warmth sensation related to C-fibre input. Warm laser stimuli were calibrated in the foot. For each subject the same laser intensity evoking a gentle, painless warmth sensation was maintained for all body sites. Ten laser stimuli were delivered. The laser beam was shifted slightly after each stimulus. The interstimulus interval was varied pseudorandomly (10–15 s). To exclude Aδ-fibre activation we concomitantly recorded EEG activity to detect Aδ-fibre related scalp potentials.

One millimetre of 3% capsaicin in a cream base (Teofarma) was applied topically with a cotton swab to a skin area measuring 1 cm2. The area of application was standardized by using a cutout to mark the area to fill with capsaicin. In all subjects, the skin area to which cap-

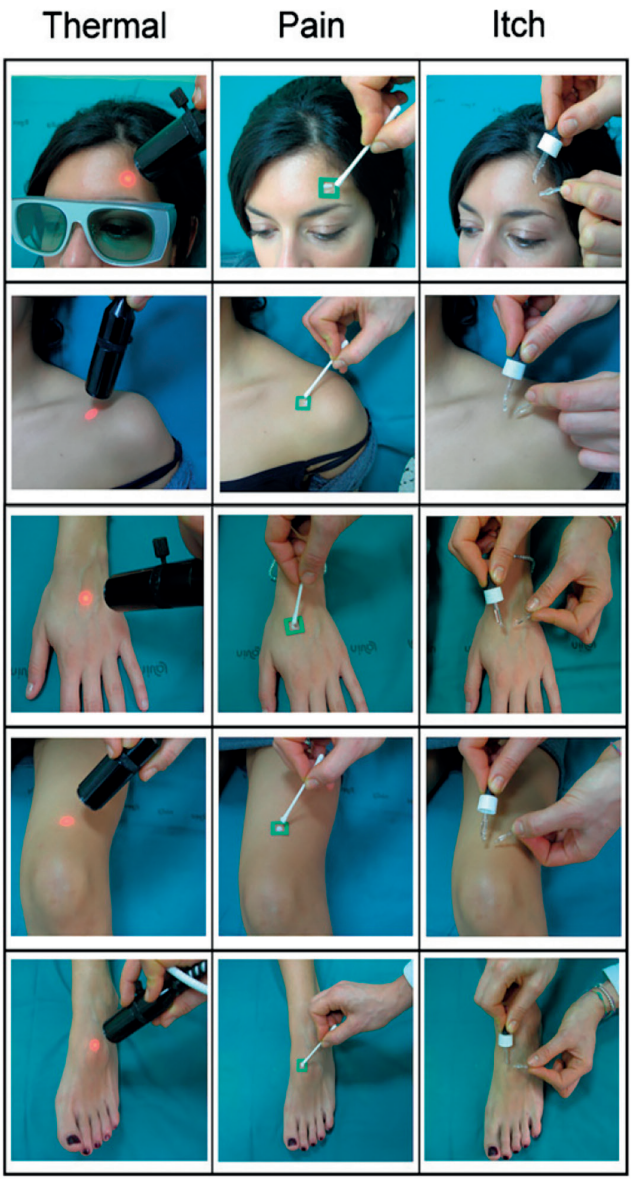

Fig. 1. Illustrative photos of laser stimulation, capsaicin cream application and histamine prick at the various body sites (face, shoulder, hand, thigh, and foot).

saicin was applied was surrounded by a halo of reactive hyperaemia (flare). The skin area outside the flare, in which mechanical stimuli elicited an evident hyperalgaesic response was considered as the zone of secondary hyperalgesia [26].

0.1% histamine was dropped onto the skin and pricked with a conventional lancet tip, as is done in standard allergy tests. In all subjects histamine prick induced a wheal and flare. The skin area that surrounded the flare, and where touching and stroking the skin lightly with a cotton swab caused itching, was considered as the zone of alloknesis [29].

Laser stimuli, capsaicin cream, and histamine prick were applied on the face (above the supraorbital notch), the shoulder (just below the acromion), the dorsum of the hand, the thigh (the skin above the knee) and the dorsum of the foot (Fig. 1) of the right side. The order of the body sites was changed randomly. The three stimulus conditions (prick histamine, warm-laser stimuli, and capsaicin) were divided in two experimental sessions on separate days. Prick histamine and warm-laser stimuli were applied in one session (about 20 min elapsed between the two stimuli conditions) and capsaicin in the other session. The order of the two sessions was randomly determined.

Subjects were instructed to rate the intensity of sensation on an 11-point numerical rating scale ranging from 0 (no sensation) to 10 (strongest imaginable sensation). The other outcome variables measured during capsaicin and histamine sessions were the longest axis of the evoked flare, the area of secondary hyperalgesia and alloknesis.

One-way analysis of variance (ANOVA) for repeated measures, post test for linear trend and the post hoc Tukey's multiple comparison test were used to analyze differences in the magnitude of thermal, pain and itch sensations at the various body sites. P values < 0.5 are considered to indicate statistical significance.

Results

In all subjects laser stimuli, capsaicin cream and histamine prick induced distinct warmth, burning, and itch sensations. No subjects reported itch after capsaicin application. The concomitant EEG recording in laser experiments showed no Aδ-fibre related neural signal, thus showing that laser stimuli activated the thermal pathway alone.

The warmth, burning, and itch sensations differed in magnitude at the various body sites ($P < 0.0001$, ANOVA). Post hoc analysis found a significant linear trend according to distance from the brain ($P < 0.0001$; ANOVA, post test for linear trend). Whereas the warmth and pain sensations became weaker and the capsaicin-induced flare and secondary hyperalgesia decreased in diameter from face to foot, the itch sensation became stronger

and the histamine-induced flare increased in diameter ($P < 0.0001$) (Fig. 2). The diameter of alloknesis showed no significant trend ($P = 0.3$).

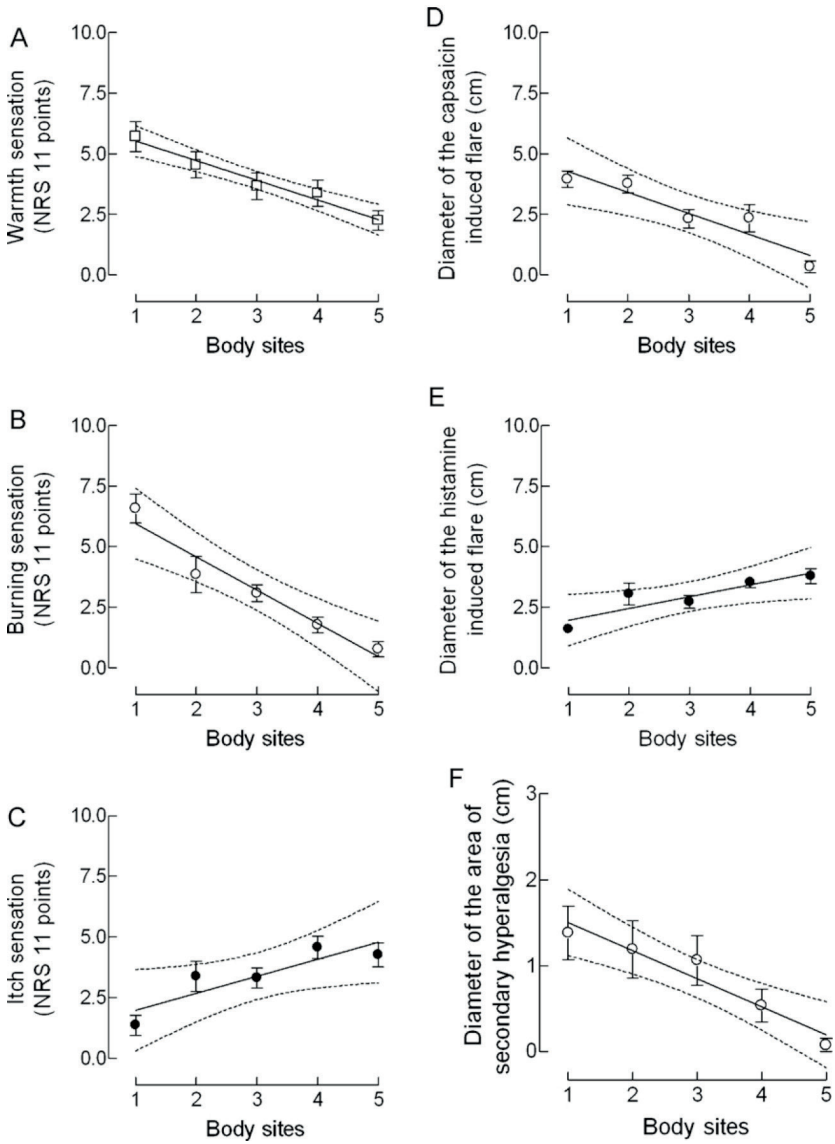

Fig. 2. Magnitude of warmth (A), burning (B), and itch sensations (C); diameter of the flare evoked by capsaicin cream application (D) and histamine prick (E); diameter of secondary hyperalgesia evoked by capsaicin cream application (F) at the various body sites, ranked according to their distance from the brain: (face: 1, shoulder: 2, hand: 3, thigh: 4, and foot: 5). Dots are the mean ± 1SE. The continuous lines indicate linear regression and dashed lines the 95% confidence limits.

Discussion

The new finding in this psychophysical study in humans is that whereas the magnitude of warmth and pain sensations is stronger at proximal than at distal body sites, itch sensation shows an opposite trend. These findings extend current knowledge on the body distribution of C-units related to sensations of warmth, pain and itch in humans suggesting that unlike thermal and pain receptors, itch receptors (pruriceptors) are denser at distal than at proximal body sites.

Our findings on the topographical distribution of warmth and burning receptors partly agree with previous findings from skin biopsy studies showing that intraepidermal nerve fibres are denser (60% higher) at proximal than at distal body sites [16]. However, skin biopsy studies do not distinguish between Aδ- and C-related receptors and the different subpopulations of C-units. By eliciting sensations with three types of stimulation we distinguished between C-units related to sensations of warmth, pain and itch.

In a previous study using a CO2 laser, we found that the threshold for pinprick sensation (Aδ-units) significantly increased with distance from brain, i.e. indicating a higher receptor density at proximal sites as found here for burning C-units; laser warmth thresholds, in contrast, did not correlate significantly with distance [1]. In that study the sensory thresholds rather than the magnitude of sensation was investigated because with the CO2-laser pulses it was more difficult to separate warmth and pinprick sensations. Furthermore it was often impossible to evoke a warm sensation at the foot, which made it far more difficult to reach a statistical significance [1]. These data suggest that the more recent YAP laser stimulator, evoking a distinct warmth sensation in all subjects, is a more adequate tool for thermal pathway assessment [5,28].

Our findings, demonstrating an opposite trend between histamine- and capsaicin-induced sensations across the different body sites, argue against possible overlap in C-units responsive to capsaicin and histamine [22,30] and indirectly suggest that histamine selectively activates a specific C-unit subpopulation. Our findings therefore agree with recent experiments in healthy subjects showing that capsaicin desensitization leaves histamine-induced itch almost unchanged [11].

In our study we investigated the body distribution of secondary hyperalgesia and alloknesis. It is largely acknowledged that secondary hyperalgesia is mediated by myelinated mechanoreceptor units, but

it requires the ongoing activity of primary afferent C-nociceptors [21]. Similarly, several studies demonstrated that ongoing activity of primary afferent C-pruriceptors evoke central sensitization of the itch pathway that manifests with touch- or brush-evoked itch around the itch site (alloknesis) [21,29]. In our study the area of secondary hyperalgesia differed between body sites at various distances from the brain, and the area of alloknesis did not. Whether this unexpected finding reflects a specific biological difference in central itch pathway organization or depends on a limitation of our experimental procedure remains unclear. Unlike secondary hyperalgesia, because the area of alloknesis is small (a diameter less than 1 cm) [9], and the intensity of itch induced by touch and brush is mild, alloknesis is difficult to measure.

It could be argued that the weaker intensity of warm and burning sensation at the distal body areas might be partly due to the signal dispersion along slow-conducting afferents such as C-fibres. However because the three sensory modalities we investigated (warmth, burning and itch sensations) are mediated by C-fibres, a homogeneous effect of the dispersion along the afferent pathways on the three sensory modalities should be expected. Thus a presumed signal dispersion along the conduction distance presumably does not change our results.

Differences in skin microvasculature, thickness of the stratum corneum and baseline skin temperature in the various body sites may affect our data. However the influence of skin microvasculature on flare should be similar for both histamine and capsaicin application (in contrast we found an opposite trend). Although the stratum corneum is thicker at sun-exposed sites [8,13], laser stimuli are not influenced by the thickness of the stratum corneum [14], and the body distribution of histamine-related itch and flare does not follow the stratum corneum thickness differences. The difference of baseline temperature is lower at the distal than at the proximal body sites, being skin temperature of the hand about 2 ◦C higher than that of the foot [2]. However this difference can hardly influence the laser- and capsaicin-induced sensations. In normal conditions the skin temperature is below the threshold for the laser warmth sensation [14] and that for activating TRPV1 channels [4].

As a previous study [15] showed that 8% capsaicin patch applied for 30 min does not significantly change epidermal nerve fibre density nor thermal detection threshold, we exclude that the 3% capsaicin we used in our experiments could induce massive axonal degeneration therefore affecting sensory perception.

We did not assess histamine-independent itch pathways. Clinical and experimental evidence shows that other mediators (e.g., cowhage spicules) elicit itch by activating C-units that do not respond to histamine [19]. A recent study in monkeys showed that cowhage activates polymodal nociceptors [12]. Our findings encourage further research designed to verify whether the topographical distribution of the psychophysical response to cowhage parallels that of histamine.

Our psychophysical study in healthy volunteers showing that the topographical distribution of pruriceptors differs from that of C-units responding to thermal stimuli and capsaicin, provides new information supporting the idea that specific unmyelinated C-units mediate sensations of warmth, burning and itch. These findings may be useful in understanding pain and itch arising from unmyelinated pathway damage.

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Study III

Peripheral nociceptor sensitization mediates allodynia in patients with distal symmetric polyneuropathy

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Introduction

In patients with peripheral neuropathy, neuropathic pain manifests with spontaneous and provoked symptoms [1]. Spontaneous symptoms include ongoing pain and paroxysmal pain, whereas provoked pain frequently manifests as dynamic mechanical allodynia, namely pain in response to normally non-painful brushing [2].

The reference standard test for diagnosing peripheral neuropathy is the nerve conduction study (NCS) [3]. NCS nevertheless has the disadvantage of assessing non-nociceptive, large-myelinated fibres (Aβ fibres) alone and provides no information on nociceptive pathway function [3]. The current neurophysiological test for assessing nociceptive pathways entails recording laser-evoked potentials (LEPs) [3, 4]. Laser-generated radiant heat pulses selectively activate Aδ and C mechano-thermal nociceptors, and evoke scalp potentials related to small myelinated (Aδ) fibres [5, 6]. LEPs are the most reliable and agreed-upon neurophysiological method for investigating nociceptive fibre function in patients with pain [6, 7].

Although patients with peripheral neuropathy frequently present with allodynia, its underlying mechanisms remain open to debate. Most investigators attribute allodynia to central mechanisms [8, 9]. In patients with peripheral neuropathy, the spontaneous firing in damaged nociceptive afferents may evoke ongoing pain and sensitise second-order neurons in the dorsal horn. Sensitised neurons respond to Aβ fibre input with high-frequency activity that the brain perceives as painful [9, 10]. An alternative and opposite view suggests that provoked pains, including allodynia, could reflect a lowered mechanical

threshold in sensitised intra- epidermal nociceptors [11, 12]. More reliable neurophysiological information on the pathophysiological mechanisms underlying allodynia related to distal symmetric polyneuropathy could be used to develop more effective therapeutic approaches for this type of pain.

We hypothesised that if Aβ-fibres do mediate allodynia, then NCS responses should detect differences in Aβ-fibre function in patients with and without this type of neuropathic pain. Hence in this prospective clinical and neurophysiological study, we recorded NCS, assessing Aβ-fibre function, and LEPs, assessing nociceptive fibres, in patients with distal symmetric polyneuropathy, with and without allodynia. As the primary outcome variables, we assessed the amplitude of the sural nerve sensory action potential, and foot-LEPs.

Methods

We prospectively collected 200 patients with distal symmetric polyneuropathy (114 with pain and 86 without). The diagnosis was based on clinical, biological, and electrodiagnostic findings, adhering to the criteria proposed by England et al. [13] (i.e., patients with symmetrically reduced or absent ankle reflexes, decreased distal sensation, and abnormal NCS or skin biopsy findings). We included patients with four different aetiologies: diabetes- related neuropathy (70), chemotherapy-induced neuropathy (53), cryoglobulin-related neuropathy (30), and neuropathy of unknown origin (47). Exclusion criteria were sensory disturbances due to neurological diseases other than distal symmetric polyneuropathy and cognitive impairment.

Two staff members examined the patients clinically, and the others did neurophysiological testing, with those recording NCS being blinded to LEP data and vice-versa. The research was approved by the Institutional Review Board and patients gave their informed consent.

All patients underwent clinical examination using bedside tools. Patients were grouped according to the clinically documented presence or absence of neuropathic pain, as assessed by the DN4 questionnaire [14]. The DN4 questionnaire is a clinician-administered screening tool that comprises various clinical items, including allodynia, and indicates neuropathic pain when the score is >4.

Patients with neuropathic pain were further divided in two groups: with and without allodynia, as assessed by the dedicated

items of the DN4 (all patients without allodynia complained of ongoing pain).

Patients underwent motor and sensory NCS testing using surface recording electrodes with standard placement. Methods used adhered to those recommended by experts of the International Federation of Clinical Neurophysiology [15]. NCS testing comprised sensory nerve action potentials (SNAP) and antidromic conduction velocities recorded from sural and superficial radial nerves, and ortodromic conduction velocity from ulnar nerve. Other nerve function variables examined were compound motor action potential (CMAP) amplitude and peroneal, tibial and ulnar nerve condition velocities. We studied LEPs using a neodym- ium:yttrium–aluminium–perovskite (Nd:YAP) laser. The dorsum of the right foot and the left hand were stimulated by laser pulses at relatively high intensity (150–200 mJ/mm2), short duration (5 ms), and small diameter (*5 mm), eliciting pinprick sensations. Subjects lay on a couch and wore protective goggles. To determine the laser perceptive threshold, we delivered a series of stimuli at increasing and decreasing intensity, and defined the perceptive threshold as the lowest intensity at which the subjects perceived at least 50 % of laser stimuli. The early, lateralized component, N1, and the main complex, N2-P2, were recorded through disc electrodes from the temporal areas (Tc) referenced to frontal area (Fz) and vertex (Cz) referenced to the nose. From 10 to 20 trials devoid of artefacts were collected and averaged offline. We measured peak latency and amplitude (peak-to- peak) of the temporal N1 component and the N2-P2 vertex complex. NCS and LEP data were compared with normative ranges established in our laboratory.

Statistical analysis

Because all patients had sensory disturbances predominantly distributed to the feet (in particular, allodynia affected feet only) we limited the statistical analysis to foot-related neurophysiological responses (see Tables 1, 2). Furthermore, only the most relevant neurophysiological variables (i.e., sural SNAP and foot-LEPs) will be presented and discussed in the text. Mann–Whitney U test was used to analyse the differences in non-normally distributed neurophysiological data (peroneal CMAP and sural SNAP, laser percep-

tive thresholds, N1 latency, N1 and N2-P2 LEP amplitude). Unpaired t- test was used to compare data with normal distribution (sensory and motor nerve conduction velocity, N2 latency). The differences in the frequency of neuropathic pain and allodynia across the different aetiologies were analysed with the v2 test. P values < 0.05 were considered to indicate significance. In the tables all results are reported as mean ± SD.

Results

Of the 200 selected patients with distal symmetric polyneuropathy (all having distal, symmetric sensory disturbances), most had a predominantly sensory neuropathy, 114 with pain and 86 without. Although clinical assessment showed that most patients, regardless of pain, had sensory deficits involving all sensory modalities, pin-

Tab. 1. Foot-related neurophysiological responses in patients with and without pain.

	Patients without pain (mean ± SD)	Patients with pain (mean ± SD)	*P*
Sural SNAP amplitude (μV)	3.6 ± 2.8	3.3 ± 4	0.14
Sural NCS (m/s)	46.7 ± 3.8	47.9 ± 4.1	0.15
Peroneal CMAP amplitude (mV)	4.2 ± 2.1	3.9 ± 2.2	0.2
Peroneal NCV (m/s)	46.2 ± 2.7	46.5 ± 3.6	0.69
Warm perceptive threshold (mJ/mm^2)	51.2 ± 12.4	59.14 ± 15.83	0.0002
Pinprick perceptive threshold (mJ/mm^2)	89.87 ± 18.42	106.9 ± 31.97	0.0005
Foot N1LEP latency (ms)	204.3 ± 38.1	208 ± 22.1	0.43
Foot N1LEP amplitude (μV)	2 ± 2.4	0.6 ± 1.5	<0.0001
Foot N2LEP latency (ms)	264.7 ± 28.5	259.3 ± 66.3	0.61
Foot N2-P2LEP amplitude (μV)	15.1 ± 10.1	7.6 ± 9.2	<0.0001

prick and warm thresholds assessed with laser stimuli were significantly higher in patients with pain than in those without ($P < 0.01$) (Table 1). In the group of patients with pain, DN4 identified 44 patients with allodynia, 70 without. V^2 test showed no differences in the frequency of pain and allodynia across the different aetiologies ($P < 0.5$). Whereas foot-LEP amplitude (the N1 component and N2-P2 complex) was significantly lower in patients with pain than in those without ($P < 0.0001$, Mann– Whitney test), neither sural SNAP ($P < 0.1$), nor LEP latency and sensory conduction velocities differed in the two groups ($P < 0.1$) (Tab. 1). The comparisons between neurophysiological responses in patients with and without allodynia (Fig. 1) showed that whereas warm and pinprick perceptive thresholds and the mean foot-LEP amplitude was higher in patients with allodynia than in those with ongoing pain alone ($P < 0.01$), the sural SNAP amplitude did not differ ($P < 0.1$) (Tab. 2; Fig. 2).

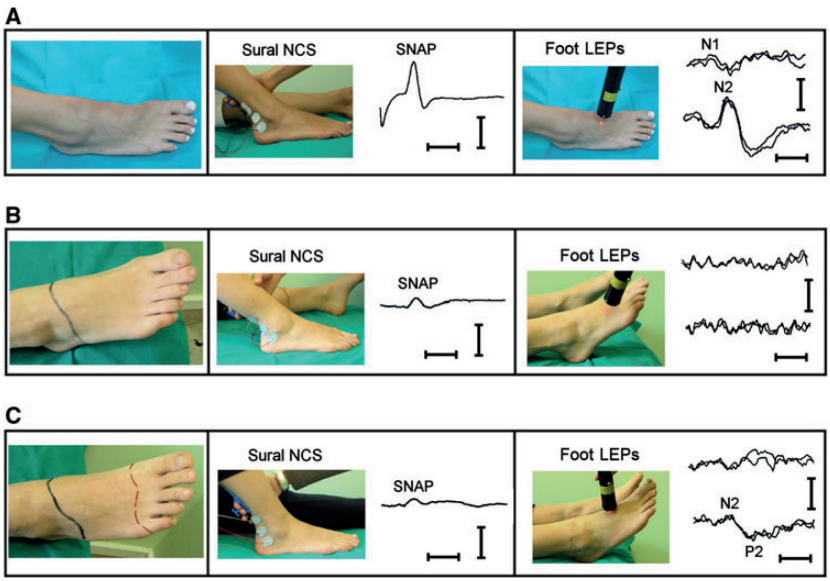

Fig. 1. Neurophysiological assessment in a control subject
(a) and in representative patients without allodynia
(b) and with allodynia (c). Figures show the distribution of pinprick hypoesthesia (black line) and allodynia (red line), and laser-evoked potentials (LEPs) and nerve conduction study (NCS) recordings. NCS data were similar in the two patients. LEPs were absent in patient without allodynia, but only partially reduced in patient with allodynia. NCS nerve conduction study. SNAP sensory nerve action potential. LEP laser evoked potentials. Horizontal calibration 2 ms for NCS, 20 ms for LEPs. Vertical calibration 10 μV

Tab. 2. Foot-related neurophysiological responses in patients with and without allodynia

	Patients without allodynia (mean ± SD)	Patients with allodynia (mean ± SD)	*P*
Sural SNAP amplitude (μV)	3.6 ± 2.9	2.9 ± 3.4	0.1
Sural NCS (m/s)	48 ± 4.1	47.6 ± 4.3	0.73
Peroneal CMAP amplitude (mV)	4.1 ± 2.2	3.3 ± 2.2	0.07
Peroneal NCV (m/s)	46.9 ± 3.3	45.8 ± 3.9	0.2
Warm perceptive threshold (mJ/mm^2)	62.48 ± 16.23	52.76 ± 13.11	0.0005
Pinprick perceptive threshold (mJ/mm^2)	116.1 ± 34.13	94.16 ± 23.29	0.001
Foot N1LEP latency (ms)	225 ± 29.7	199.3 ± 10.6	0.1
Foot N1LEP amplitude (μV)	0.13 ± 0.6	1.7 ± 2.3	0.001
Foot N2LEP latency (ms)	258 ± 28.8	258 ± 28.3	1
Foot N2-P2LEP amplitude (μV)	5.4 ± 7.6	11 ± 10.6	0.007

Discussion

In our prospective clinical and neurophysiological study in a large cohort of patients with distal symmetric polyneuropathy, NCS testing failed to detect differences in Aβ-fibre function in patients with and without allodynia. Conversely, LEP recordings showed larger-amplitude foot-LEPs in patients with allodynia than in those without. These findings imply that Aβ-fibres have no role in mediating allodynia in patients with distal symmetric polyneuropathy and suggest that this type of pain might be associated with partially preserved and sensitised nociceptive nerve terminals.

In our group of patients with distal symmetric polyneuropathy, we found no differences in pain frequencies, or LEP or NCS abnormalities, according to aetiology. This finding is in line with previous studies that found no association between neuropathic pain disorders and aetiology [16, 17]. Hence, we believe that studies seeking information on

pain mechanisms should group patients according to clinical features rather than aetiology.

When we investigated neurophysiological differences between patients with and without pain, we found that whereas LEPs had significantly smaller amplitudes in patients with pain, NCS data were similar in the two groups. These findings support the current knowledge on neuropathic pain. Previous clinical, neurophysiological, and neuropathological investigations showed that in patients with peripheral neuropathy of various aetiologies, neuropathic pain is invariably associated with nociceptive pathway damage [17–20].

When we compared neurophysiological data in patients with and without allodynia, we found no differences in NCS data in the two groups. The lack of differences in Aβ-fibre- mediated NCS between patients with and without allodynia suggests that in most patients second-order neuron sensitisation to Aβ-fibre input might be unnecessary for the development of allodynia. Although we found reduced-amplitude LEPs in patients with allodynia, the LEP attenuation was significantly lower than that in patients with painful neuropathy without this type of pain. This finding indicates that in patients with allodynia, nociceptive afferents are partially spared, and also suggests that allodynia might reflect a lowered mechanical threshold in intraepidermal nociceptive nerve terminals.

Over the past decades, ample evidence underlines a possible role for sensitised nociceptive terminals as primary determinants of pain in humans [21–23]. Previous studies directly demonstrated reduced C nociceptor thresholds to mechanical stimuli in humans with provoked pain [12]. In patients with postherpetic neuralgia, many studies showed that allodynia correlates with temperature sensation sparing, thus suggesting the need for a relative temperature-pain afferent fibre sparing [24]. Support for peripheral nociceptor sensitisation as the main mechanism responsible for allodynia also comes from placebo controlled trials showing that topical lidocaine, a drug that selectively blocks Aδ and C fibres, reduces allodynia [25, 26]. Some studies dealing with the mechanisms underlying allodynia distinguished patients with neuropathic pain according to their cutaneous nociceptor function: in some patients, ongoing pain and allodynia arise from sensitised nociceptors, in others there is a massive loss of cutaneous nociceptors within the allodynic skin [27]. These data raise the possibility that in patients with cutaneous nociceptor loss allodynia is mediated by Aβ-fibres. In our study we could not distinguish between patients with preserved and im-

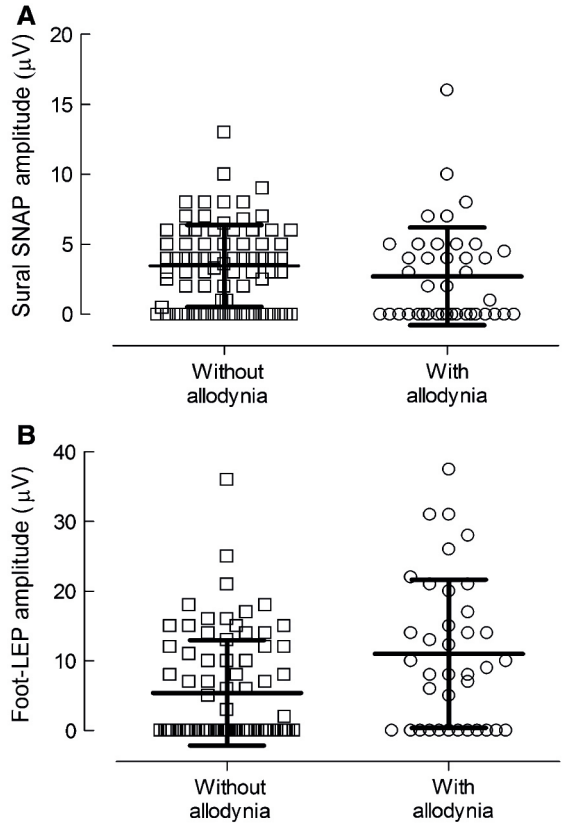

Fig. 2. Statistical analysis in patients with (n = 44) and without allodynia (n = 70). While sural sensory nerve action potential does not differ between patients with and without allodynia (a), the amplitude of the N2-P2 complex of the laser evoked potentials after foot stimulation is higher in patients with allodynia than in those without (b)($P < 0.01$, Mann–Whitney test)

paired cutaneous nociceptors, because all our patients had thermal pain sensory deficits, though variable in severity. This limitation notwithstanding, a previous study [28] showed that lidocaine, a drug that selectively blocks cutaneous nociceptors [26], effectively relieved allodynia also in patients with impaired nociceptor function, thus suggesting that even in this group of patients allodynia might be mediated by the few surviving and sensitised nociceptors. The need of partially preserved nociceptive nerve terminals for developing allodynia implies that a massive loss of peripheral nociceptors causing severe sensory deficits may prevent the development of allodynia. Hence in patients with distal symmetric peripheral neuropathy, allodynia might predominantly manifest at the initial or mild stages of peripheral neuropathy when

cutaneous nociceptive nerve terminals are still partially spared. We also hypothesize that a minimal critical number of sensitized nociceptors is probably needed for developing allodynia. Further skin biopsy studies assessing intraepidermal innervation might clarify this point. Some limitations regarding the present study should be addressed. We found significant differences in group analysis, but we cannot provide reliable conclusions on mechanisms underlying allodynia in single patient. We also cannot exclude the possibility that allodynia might occasionally develop partly through central mechanisms. Spared nociceptors might sensitize second-order neurons to Aβ-fibre input, thus producing allodynia. Many animal studies directly proved second-order neuron sensitisation to Aβ-fibre input, and some clinical observations in healthy humans and patients indicate that a selective Aβ-fibre block reduces allodynia [29, 30]. Notwithstanding these possible limitations, our study reliably demonstrates that a better preserved nociceptor population in neuropathy increases the risk of developing allodynia. This suggests that second-order neuron sensitisation to Aβ-fibre input might be unnecessary for the development of allodynia. The information from our study showing that allodynia in patients with distal symmetric polyneuropathy is associated with partially preserved nociceptive afferent fibres and possibly unrelated to Aβ-fibres could be useful in designing new treatment strategies targeted to this type of pain.

Ethical standard All human studies must that they have been approved by the appropriate ethics committee and therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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Study IV

Breaking dogmas: paroxysmal pain is mediated by non nocipeptive large myelinated fibres. A neurophysiological study in healthy subjects.

Introduction

Background

What is Neuropathic Pain?

The International Association for the Study of Pain defines neuropathic pain as pain caused by a lesion or disease of the somatosensory nervous system.[1]

Neuropathic pain is a frequent problem in many peripheral nervous system diseases, especially distal symmetrical peripheral neuropathies (such as diabetic neuropathy) and focal neuropathies related to trauma (such as traumatic brachial plexus injuries), and following surgical interventions (such as breast surgery). Central nervous system diseases that commonly cause neuropathic pain include multiple sclerosis (MS), spinal cord injury and stroke. This wide-ranging aetiology explains the high prevalence of neuropathic pain in the general population: results from postal surveys to investigate chronic pain with neuropathic characteristics in large community samples indicate that neuropathic pain has a 7–8% prevalence, and confers a tremendous medical, social and economic burden.[2]

Mechanisms based approach

Patients with neuropathic pain usually complain of a various combination of different pain symptoms. Neuropathic pain typically manifests with continuous pain (burning, squeezing, pressure) or paroxys-

mal pain (electric shock-like sensations, stabbing pain), and provoked (brush-evoked, pressure-evoked, cold-evoked), or paraesthetic and dysaesthesic (tingling, pins and needles) sensations.[4]

Although in some etiologic categories of neuropathic pain specific types of pain may predominate, none of them are etiologic specific. Hence, patients suffering from an identical disease may present with heterogeneous sensory signs and symptoms. The diagnostic workup should therefore aim to define specific sensory profiles through clinical examination, questionnaires dedicated to neuropathic pain, and laboratory tools. Neuropathic pain can now be classified by sensory profiles (quality of pain) rather than aetiology, as the recent European guidelines recommend.[5] Classifying neuropathic pain according to a mechanism-based rather than an aetiology-based approach might minimize pathophysiological heterogeneity within the groups under study and thus help in targeting therapy to the individual patient. It would also be useful in testing new drugs.

Types of spontaneous neuropathic pain

Although neuropathic pain frequently manifests with a combination of spontaneous and provoked pains, in most clinical conditions, spontaneous pain usually represents the predominant complaint of patients suffering from neuropathic pain [6]. The most typical quality of spontaneous pain is the ongoing burning pain. Clinical studies report that the frequency of burning pain ranges from 51% to 90% in patients with peripheral neuropathy [6]. Most clinical and neurophysiological studies showed that this type of pain is associated with nociceptive pathway damage. More specifically neurophysiological studies have shown that in patients with neuropathic pain related to peripheral and central nervous system diseases (postherpetic neuralgia, carpal tunnel syndrome, polyneuropathy and multiple sclerosis), the severity of ongoing burning pain is inversely related to the amplitude of nociceptive-mediated laser evoked potentials. This relationship—although only an indirect finding in some instances—indicates that ongoing burning pain is strongly associated with damage to the nociceptive system.[7][8][9]

Insofar as dysfunction of pain pathways results in painful sensations, and evidence in animal models and humans shows damage to pain pathways in neuropathic pain conditions, expert opinion is that neuropathic pain invariably arises from damage to nociceptive rather than non-nociceptive pathways.[10]

However in patients with postherpetic neuralgia and carpal tunnel syndrome, paroxysmal electric-shock-like pain is associated with neurophysiological abnormalities involving non-nociceptive Aβ-fibres. More specifically, in patients with postherpetic neuralgia and carpal tunnel syndrome the correlation between the delayed responses to blink reflex testing and median-nerve sensory conduction velocity slowing and paroxysmal electrical shock-like pain suggests that this type of pain is related to focal Aβ-fibre demyelination.[7][8] In patients with multiple sclerosis, Lhermitte phenomenon (the classic electrical shock-like sensation) is associated with abnormalities in dorsal column-mediated somatosensory evoked potentials, whereas spinothalamic-mediated laser evoked potentials are spared. This finding suggests that Lhermitte phenomenon originates from demyelination in non-nociceptive dorsal columns, and leaves the nociceptive spinothalamic pathways almost intact.[9]

The possibility of a correlation between the electric-shock-like pain and demyelination of (non-nociceptive) large myelinated fibres is indirectly supported by numerous observations that trigeminal neuralgia—the neuropathic pain condition that most typically causes paroxysmal electric-shock-like pain—is due to focal demyelination that mainly affects large myelinated fibres [11]. In classic trigeminal neuralgia (caused by compression of the trigeminal root by aberrant vessels) and symptomatic trigeminal neuralgia (caused by compression or stretching of the trigeminal route by slow-growing benign tumours), the focal compression mechanically damages large myelinated fibres, causing demyelination. In animal and human experimental studies, nerve compression (by a weight or a pneumatic cuff) injures large-myelinated fibres, whereas small myelinated and unmyelinated fibres remain unaffected. Symptomatic trigeminal neuralgia due to a pontine demyelinating plaque related to multiple sclerosis directly arises from demyelination of primary afferent fibres. Whether produced by chronic compression or multiple sclerosis, demyelination of Aβ-fibres increases the susceptibility of these neurons to ectopic excitation and high-frequency discharges, producing typical paroxysmal pain. According to the proposed mechanism for electric-shock-like pain, the most appropriate treatments are probably carbamazepine and oxcarbazepine, as such agents produce a frequency-dependent block of voltage-gated sodium channels and, thereby, reduce the frequency of action potential firing. This assumption is supported by the evidence that these drugs produce considerable pain relief in most patients with trigeminal neuralgia.

Moving from this assumption we devised a neurophysiological study aiming at verifying whether high-frequency electrical stimulation selectively activating intact Aβ-fibres can produce paroxysmal pain in healthy subjects. To do so we investigated six patients with typical Trigeminal Neuralgia (TN) by delivering a train of stimuli at their fifth digit using various frequencies (100-200 Hz), durations (0.5-1 sec) and intensities lower than pain threshold when delivered in single pulses, to ascertain which kind of stimulus mostly approached the painful paroxysm occurring during a TN attack and we therefore reproduced it in 10 normal subjects before and after a ropivacaine blockade, selectively inactivating small diameter fibres.

Preliminary experiment

Healthy Subjects:

Psychophysical test

We performed in 10 healthy subjects aged 24-33 years (mean 28 years; 4 males, 6 females) a psychophysical test to ascertain which kind of stimulus was able to evoke a shock-like painful paroxysm. Each subject underwent 24 different stimulations with randomized different intensities (1.5, 2 and 2.5 times the perceptive threshold) ,frequencies (10 Hz, 50 Hz, 100 Hz, 200 Hz) and durations (0.5 sec, 1 sec). After each stimulation we asked subjects to classify the perception as pain, unpleasantness or tactile and to rate it using a numerical rating scale from 0 to 10. We therefore used the setting that obtained the highest rate among the pain/unpleasant sensation (100 Hz, 1 sec, 2.5 times the perceptive threshold) as shown in Fig. 1.

Patients

To further validate data obtained in healthy subjects we recruited six patients with Trigeminal Neuralgia aged 59-74 (mean 69; 3 male, 3 female). Inclusion criteria was diagnosis of typical trigeminal neuralgia, i.e. episodic, unilateral, lancinating, triggerable, often shock-like facial pains and pain-free intervals [12]. We tested their perceptive threshold by delivering a single pulse stimulus at increasing intensities, subsequently we performed a train of stimuli at different frequencies (100,200 Hz) and different durations (0,5;1 sec), using an

intensity twice and a half the perceptive threshold in single pulse to be sure not to activate anything but the large myelinated fibres. We therefore asked patients to compare our bursts to the one occurring during an attack of Trigeminal Neuralgia and to indicate the most like. Once we obtained the settings to deliver an high frequency, low intensity train of stimuli (100Hz, 1 sec, NRS mean 5,75±1,25) very similar to Trigeminal neuralgia paroxysm (although all patients said

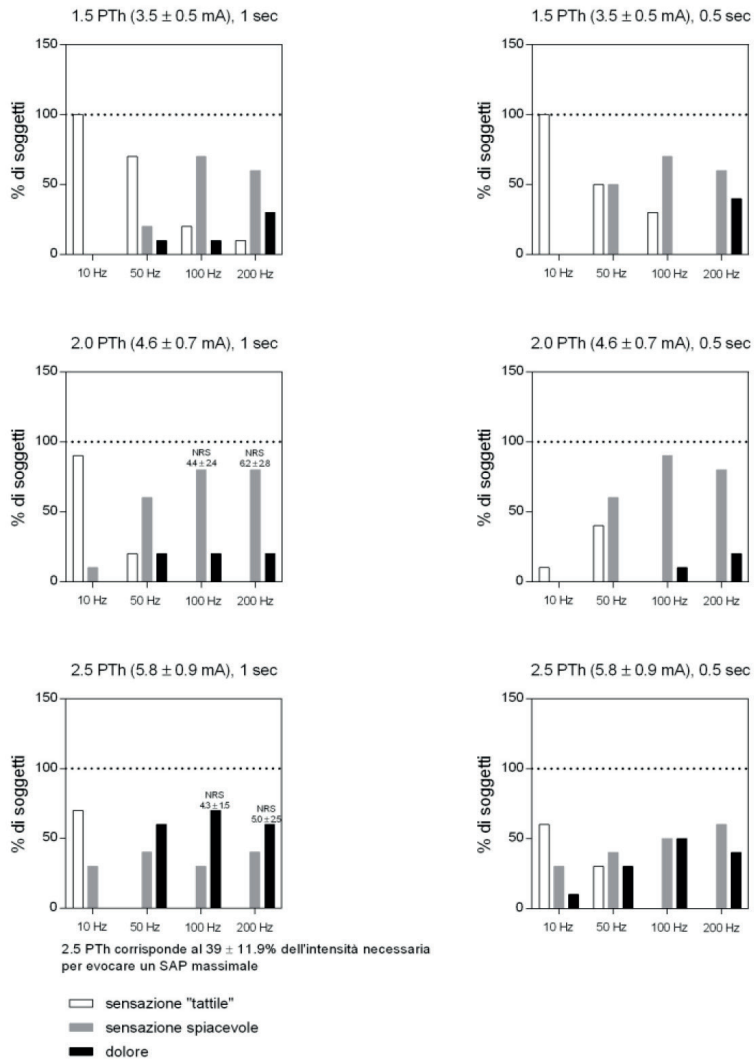

Fig. 1. x axis: number of subjects; y axis: different frequencies in Hz (10, 50, 100, 200) white: tactile; grey: unpleasantness; black: pain.

that their paroxysms lasted more), we performed the experiment in 10 healthy subjects.

NRS values assigned to different trains of stimuli by each patient are resumed in Tab. 1.

	200 hz 1 sec	**200hz 0,5 sec**	**100hz 1 sec**	**100hz 0,5 sec**
NRS Patient 1	10	7	5	7
NRS Patient 2	6,5	6,5	6,5	6,5
NRS Patient 3	6	7,5	7,5	5
NRS Patient 4	2,5	3,5	3,5	0
NRS Patient 5	8	8	6	6
NRS Patient 6	0	0	6	0
Mean±SD	**5,5±3,3**	**5,4±2,8**	**5,75±1,25**	**4±2,9**

Tab. 1. NRS values assigned to different trains of stimuli by each patient.

Material and methods

We studied 10 subjects aged 24-33 years (mean 28 years; 4 males, 6 females). First in the ulnar nerve territory of all subjects we assessed the mechanical detection threshold with a standardized set of von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany, 0.25 and 512 mN graded by a factor of 2); subsequently we evaluated the thermal thresholds, using a TSA- II Neuro-sensory analyzer: Peltier thermode 30x30mm size, TSA-II S/ 0ºC – 50ºC. (Medoc, Israel). Using the method of limits, cold detection threshold (CDT),warm detection threshold (WDT), cold pain threshold (CPT) and warm pain threshold (WPT) were determined over three consecutive trials (baseline temperature of the thermode was 32° C, upper limit 50° C and lower limit 0° C). Ulnar nerve sensory conduction was recorded orthodromically with ring electrodes placed at the fifth digit and amplitude of sensory nerve action potential recorded 2 cm proximal to the distal wrist crease. At least we detected pinprick threshold with a neodymium:yttrium–aluminium– perovskite (Nd:YAP) laser (wavelength 1.34 mm, pulse duration 2–20 ms, maximum energy 7 J) and we therefore recorded laser evoked potentials from the same territory (the early, lateralized component, N1, and the main complex, N2–P2, were recorded through disc electrodes from the temporal areas (Tc) referenced to frontal area (Fz) and vertex (Cz) referenced to the nose; from 10 to 20 artefact-free trials were collected and averaged), using an inten-

sity of stimulation equal to each subject pain tolerance (i.e. the highest intensity of laser stimulation each subject could tolerate). We considered worthwhile to introduce pain tolerance as a limit value for laser stimulation to prevent cutaneous lesions, surely occurring by increasing laser intensity during pinprick threshold evaluation after ropivacaine blockade. Subsequently we performed an high frequency (100Hz, 1 sec) low intensity (i.e. twice and half the perceptive threshold delivered in single pulses) train of stimuli (stimulus duration 0.1 msec) using ring electrodes at the fifth digit and ground electrode on the hand palm. We asked each subject to report the pain score on a 0-10 NRS.

Once we obtained basal values, we performed a ropivacaine block of the ulnar nerve at the wrist. We inserted a needle electrode (Botox needle) between flexor carpi ulnaris tendon and ulnar artery and set the nerve stimulator at 0.5 mA, once we obtained muscle twitches in the flexor digiti minimi brevis we injected up to 1,5 ml of ropivacaine (7,5 mg/ml) near nerve, exploiting the same needle we used for stimulation. We therefore asked subjects to stay for a few minutes with hand dangling. Thanks to ropivacaine slow onset and low potency, resulting in a marked differential blockade, we could easily test the progressive inactivation of different fibre groups, starting from small to large diameter fibres [13]. Once our subjects felt numbness in the ulnar territory, we repeated the same experiments performed before block: mechanical detection threshold, thermal and pinprick thresh-

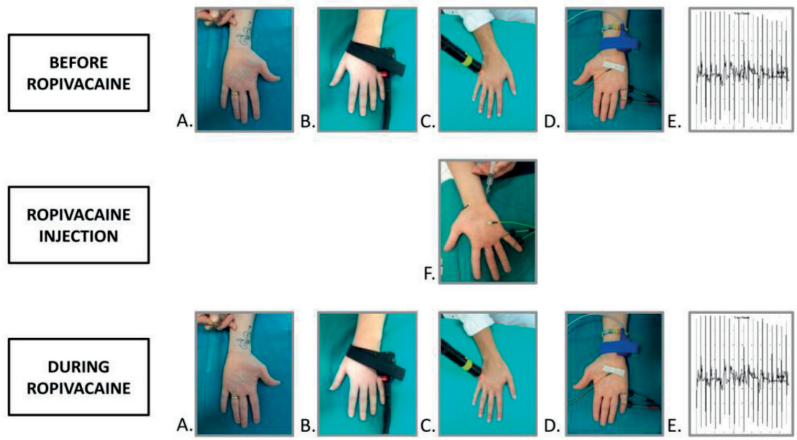

Fig. 2. Materials and methods: A. Mechanical detection; B. Thermal threshold; C. Pinprick threshold and pain tolerance D-E. Erogation of an high frequency and low intensity train of stimuli; F. Ropivacaine injection.

old, pain tolerance and Leps . Once we assessed the absence of thermal and pinprick threshold and laser evoked potentials (obtained by stimulating at pain tolerance intensity), thus confirming the effectiveness of ropivacaine blockade, we performed the same high frequency and low intensity train of stimuli and asked subjects to report the pain score on a 0-10 NRS.

Statistical analysis

We used the Kolmogorov-Smirnov test to assess the normal distribution. Paired t-test was used to analyze data normally distributed, before and after ropivacaine blockade.

The Wilcoxon matched -pair test was used for Leps amplitude which did not show a normal distribution.

$P<0.01$ was considered significant. All results are reported as Mean ± SD.

Results

Each subject showed basal values for mechanical, thermal and pain thresholds among normative range for age. (Tab. 2)

All subjects experienced paroxysmal pain during the high frequency, low intensity train of stimuli (NRS mean 5,9±0,8).

During ropivacaine blockade in the totality of subjects: mechanical thresholds remained unvaried (p=1); thermal and pain thresholds, assessed with quantitative sensory testing method of limits (WT, CT,

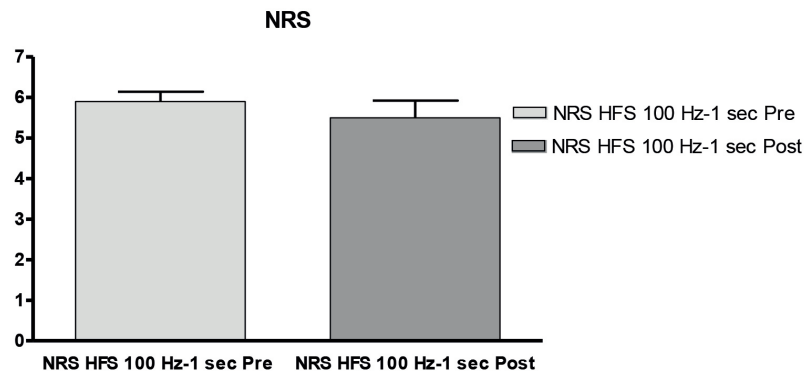

Fig. 3. Numerical rating scale (NRS) reported for high frequency train of stimuli (HFS), before (pre) and during (post) ropivacaine blockade.

WPT, CPT) were impossible to define; pinprick threshold and Laser evoked potentials, explored at increasing intensities until the pain tolerance, were absent (Tab. 2).

Numerical rating scale (NRS) reported by subjects did not significantly differ before and during complete anesthetic block of small fibres (5.9 ±0.8 and 5.5 ±1.3; P> 0.20) (Fig. 3).

	Von Frey		WT		CT		WPT		CPT		Pinprick		PT		N2-P2 A	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Subject 1	0,2	0,2	36,6	A	26,5	A	46,2	A	14,8	A	89	A	140	A	68	A
Subject 2	0,5	0,5	33,7	A	30,1	A	40,1	A	20,1	A	89	A	140	A	26	A
Subject 3	0,5	0,5	34,5	A	29,7	A	41,7	A	12,6	A	89	A	102	A	39	A
Subject 4	0,5	0,5	35,3	A	25,5	A	47,3	A	8	A	76	A	127	A	23	A
Subject 5	0,5	0,5	37,7	A	24,5	A	45,2	A	6,7	A	89	A	140	A	73	A
Subject 6	0,2	0,2	35,7	A	27,2	A	44,1	A	13,2	A	76	A	140	A	40	A
Subject 7	0,2	0,2	33,6	A	29,8	A	45,4	A	9	A	76	A	140	A	35	A
Subject 8	0,2	0,2	36,5	A	24,6	A	47,2	A	6,8	A	89	A	140	A	57	A
Subject 9	0,2	0,2	37	A	24,3	A	48,2	A	11,2	A	76	A	140	A	57	A
Subject10	0,2	0,2	36,2	A	26,3	A	42,4	A	10,7	A	64	A	127	A	52	A

Tab. 2. Von Frey: mechanical threshold values (mN); WT: warm threshold (°C) ; CT: cold threshold(°C); WPT: warm pain threshold(°C); CPT: cold pain threshold(°C); Pinprick: pinprick threshold (J/mm^3); PT: pain tolerance (mJ/mm^2); N2-P2 A: peak to peak amplitude of N2-P2 complex (μV). Each value is reported before (pre) and during (post) ropivacaine blockade.

Discussion

In this study we were able to reproduce the electric shock like sensation with defined characteristics of brevity, rapidity and suddenness, very similar to the one experienced by our patients affected by trigeminal neuralgia. This sensation arose from a selective stimulation of large myelinated non nociceptive Aβ-fibres and was considered painful or strongly unpleasant (NRS mean 5,9±0,8) by the totality of our healthy subjects, thus breaking the dogma that only nociceptive fibres can mediate pain.

The same painful/ unpleasant sensation persisted after ropivacaine nerve block of nociceptive fibres, demonstrated by the complete absence of thermal and pinprick threshold and laser evoked potentials. In our opinion this two considerations are strong enough to conclude

that Aβ-fibres surely play an important role in mediating paroxysmal pain, thus calling for a change in established knowledge, still postulating that spontaneous pain is invariably mediated by nociceptive pathways.

This data are in line with previous animal studies by Burchiel [14] describing spontaneous ectopic discharges recorded in Ab-fibre axons of cats after nerve injuries and Rasminsky [15] demonstrating that ectopic Action Potentials can be generated from areas of demyelination in peripheral nerves of dystrophic mice.

Furthermore these data are in line with our previous neurophysiological studies in patients with postherpetic neuralgia, carpal tunnel syndrome and multiple sclerosis showing that while ongoing burning pain correlated with abnormalities of nociceptive fibre-mediated LEPs, paroxysmal electric-shock-like pain was associated with neurophysiological abnormalities involving non-nociceptive Aβ-fibres [7][8][9]. The possibility of a correlation between the paroxysmal electric-shock-like pain and demyelination of (non-nociceptive) large myelinated fibres is indirectly supported by numerous observations that trigeminal neuralgia—the neuropathic pain condition that most typically causes paroxysmal electric-shock-like pain—is due to focal demyelination that mainly affects large myelinated fibres [12]. Demyelination of Aβ fibres probably increases the susceptibility of these neurons to ectopic excitation and high-frequency discharges, producing typical paroxysmal pain.

A limit of our neurophysiological experiment is that we cannot demonstrate whether Aβ-fibre activation are sufficient to provoke pain per se or rather they indirectly produce pain by depolarizing dorsal horn nociceptive C-fibre terminals [16]. However, this plausible involvement of nociceptive pathway does not hinder the key role of Aβ-fibres in mediating paroxysmal pain.

Another limit of this study is the variability among subjects in defining the high frequency burst. During preliminary test some subjects (N=3) rated the sensation as unpleasant and not painful.

This study, together with previous neurophysiological studies by our group, provide extremely convincing data about the correlation between Aβ fibers and paroxysmal pain in different pathological conditions and lead us to believe that different types of neuropathic pain are invariably caused by similar mechanisms, regardless of the underlying disease . This acquisition is based on the so-called

"mechanism-based" approach, which according to the recommendations of the most recent guidelines on the management of neuropathic pain [5] should become the cornerstone of clinical assessment and therapeutic interventions in this regard. In the present case, the supposed mechanism underlying paroxysmal pain makes reason of the effectiveness of sodium channel blockers, drugs that are successfully used in every case of electric shock like pain, regardless of aethiology. We expect that our new findings might be useful in drug trials and in tailoring therapy to the individual patient, suffering from this type of pain.

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Neuropathic pain is a common problem in clinical practice, which affects patients quality of life. The more recent approach to this peculiar type of pain is based on the "sensory profiles theory". According to this theory, neuropathic pain manifests with different combinations of sensory abnormalities, which in turn arise through different pathophysiological mechanisms. Convincing evidence now suggests that the classification of neuropathic pain according to a mechanism-based approach rather than etiology could help in targeting the therapy for the individual patient and would be useful for testing new drugs. My work has therefore focused on disclosing the pathophysiological mechanisms underlying neuropathic pain and how they translate into symptoms.

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