Temperature-dependent double spikes in C-nociceptors of neuropathic pain patients

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Five patients with small-fibre neuropathy characterized by temperature-dependent spontaneous pain, hyperalgesia/allodynia and signs of neurogenic inflammation were studied clinically and thermographically, and by microneurography. Thermography revealed hyperthermia confined to painful and hyperalgesic skin of distal extremities, in absence of sympathetic vasomotor denervation. Quantitative sensory testing documented either reduced thresholds or increased suprathreshold magnitude for heat pain. Microneurography identified 13 primary cutaneous C-nociceptors generating abnormal impulses in response to electrical stimuli and, in one patient, nociceptors firing spontaneously. All five patients showed examples of double spikes, in which a single brief electrical stimulus occasionally or regularly evoked two impulses. In one case, a second impulse occurred at one of three different delays. In all five patients, warming of the skin increased the probability of a second impulse occurring. Impulse doubling has previously been reported as occurring rarely in normal subjects and is attributable to unfiltering of multiple orthodromic impulses due to unidirectional conduction failure at branch points. A higher incidence of double firing in neuropathic pain patients is probably due to a reduced safety factor for conduction in the terminal arborizations of their C-nociceptors. These observations show that unidirectional conduction block provides a peripheral mechanism of temperature-dependent nociceptor hyperactivity in small-fibre neuropathy that may contribute to hyperalgesia.

Keywords: Hyperalgesia; microneurography; C fibre; nociceptor; neuropathic pain

Abbreviations: NCS = nerve conduction studies; QST = quantitative sensory thermotest

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Introduction

Unmyelinated (C-) nociceptor axons branch extensively in human skin to innervate cutaneous areas of approximately one to several square centimetres (Messlinger, 1996; Schmidt *et al.*, 1997, 2002). An orthodromic impulse activated in one branch normally invades every other branch antidromically. In a subset of nociceptors, the antidromic impulses produce an axon reflex flare that covers an area corresponding to the receptive field of the axon (Gee *et al.*, 1997). If a stimulus excites single impulses in multiple branches, then only the leading impulse reaches the stem axon and the CNS; the others are blocked by collision with the antidromic axon 'reflections' of the leading impulse. Thus, microneurographic recording from single C-nociceptors normally shows a single impulse in response to a single electrical stimulus to the receptive field, even though several branches may have been excited. The excitation of additional branches may, however, be revealed by jumps in latency between two or more values, caused by changes in stimulus intensity or by activity-dependent changes in excitability (Torebjörk and Hallin, 1974). During repetitive electrical stimulation, which causes activity-dependent hyperpolarization that increases threshold and slows conduction, latency jumps often occur to a longer latency, as activation at the shortest latency branch fails, allowing impulses from a slower branch to reach the parent axon (Serra *et al.*, 1999). Two latencies may alternate for a while, each showing a parallel profile of activity-dependent slowing, unaffected by which branch was excited by the stimulus. The slowing is similar in the two branches because both are eventually invaded once for each stimulus, independent of the direction of impulse propagation (Serra *et al.*, 1999).

Although orthodromic impulses normally seem to invade all branches of a C-nociceptor unit, irrespective of where they originate, Weidner *et al.* (2003) reported that this is not always the case. They found eight units in normal subjects, which sometimes conducted two impulses following a single brief stimulus and, when they did so, the profile of activity-dependent slowing was appropriate for two impulses rather than one. They argued that the two impulses originated from different terminal branches and required unidirectional conduction block to prevent the faster action potential from invading and resetting the slower-conducting terminal branch. Therefore, conduction failure in one direction at a branch point paradoxically resulted in an extra impulse reaching the CNS and constitutes a peripheral mechanism for pain amplification.

Weidner *et al.* (2003) reported that their eight units exhibiting unidirectional conduction block were found amongst recordings from ~250 C units, an incidence of roughly 3.2%. In our experience of recordings from normal subjects, we would estimate the incidence at an even lower rate and, therefore, conclude that it is normally a rare phenomenon. However, we now report 13 instances in recordings from five patients with neuropathic pain associated with small-fibre neuropathy. Moreover, in each subject we altered skin temperature and found that impulse doubling was promoted by warming the skin. We therefore suggest that this phenomenon is pathological, rather than normal and rare, and may contribute to hyperalgesia in small-fibre neuropathy.

Case reports

Case 1

L.F., a 14-year-old Canadian girl, developed a subacute painful neuropathic syndrome post-flu, featuring burning pain in the feet (and less so in the hands), severe heat and mechanical hyperalgesia relieved by passive cooling, and signs of cutaneous inflammation. Large calibre sensory and motor nerve functions were intact. Thermography displayed striking hyperthermia of the symptomatic feet. Quantitative sensory thermotest (QST) revealed reduced threshold for heat pain in the hands, increased suprathreshold magnitude of heat pain in the feet, and mild loss of warm sensation in the feet. A probable autoimmune disorder was diagnosed and, after treatment with intravenous immunoglobulin, pain gradually diminished and narcotics were withdrawn. On follow-up 4 months later, the patient was asymptomatic except for minor heat hyperalgesia in the hands.

Case 2

M.R., a 66-year-old pre-diabetic (HbA1C = 6.2%), complained of progressive spontaneous pain and hypersensitivity to mechanical contact of her recently acquired hot feet, associated with mild loss of tactile sensation. She reported moderate symptom relief with passive cooling. Neurologically, she had static mechanical hyperalgesia/allodynia in both feet, on a background of mild hypoaesthesia to light touch, pinprick, cold sensation and warm sensation. Motor function was normal and tendon reflexes preserved. Nerve conduction studies (NCS) revealed mild distal loss of motor axons only, but electromyography showed no signs of muscle denervation. Thermography displayed abnormal hyperthermia of her feet, and QST showed mildy elevated threshold for warm sensation, moderately reduced threshold for cold pain, and increased suprathreshold magnitude of heat pain.

Case 3

W.D., a 40-year-old woman, developed progressive burning pain in her feet, made worse by standing and by warming, and dramatically improved by cooling the skin. The pain ascended from toes up to ankles over a period of months. Podiatry promoted 'reflex sympathetic dystrophy' but sympathetic blocks worsened her pain, as they caused vasodilatation and warming of the skin. On examination, there was mild loss of sensation in stocking distribution. NCS revealed normal motor and sensory velocities and compound action potential amplitudes. Thermography displayed abnormal hyperthermia of her feet after walking for a while and QST documented heat hyperalgesia. A dedicated search for aetiology of the small-fibre neuropathy was unrewarding. Her diabetic mother had a similar disorder.

Case 4

R.M., an 82-year-old woman, developed cryptogenic spontaneous pain in her feet (soles) 5 years previously, associated with purplish, red, hot feet. Even the gentlest contact elicited pain. Symptoms were improved by passive cooling. All sensory and vasomotor symptoms worsened with time and spread to involve all four limbs and the whole body. The hyperalgesia/allodynia was soothed by silk or satin clothes and bed covers. There was no sensory or motor deficit and tendon reflexes were normal. Laboratory tests were negative. Thermography was variable. NCS showed normal sensory nerve action potential (SNAP) amplitudes in sural nerves for her age. QST revealed abnormally reduced thresholds for heat pain in upper and lower limbs, and elevated warm specific thresholds. All attempts at symptomatic treatment have so far failed.

Case 5

S.T., a 34-year-old woman, had a 6-month history of spontaneous pain and mechanical hyperalgesia in stocking distribution, worsened by heat, but without evident hyperthermia. She also complained of tingling in the feet and hands, and dryness of eyes and mouth. Achilles tendon reflexes were absent and there was stocking hypoaesthesia with mechanical hyperalgesia in the feet. NCS showed absence of sensory nerve action potentials in sural nerves with normal motor conduction in peroneal nerves. QST revealed cold and warm hypoaesthesia in the feet, without reduction in heat pain threshold. The diagnosis of large and small-fibre

polyneuropathy associated with primary Sjögren's disease was made. The patient improved with steroids and azathioprine, but she discontinued treatment and the painful symptoms worsened.

Following standard clinical characterization, the patients underwent one or more sessions of microneurography after signing informed consent, and as approved by the Legacy Health Systems Institutional Review Board.

Methods

Thermography

Infrared telethermography was performed using a Flexitherm Mark V device (Flexitherm, Westbury, New York, USA). Skin temperature was adapted by having patients stay at least 1 h in an air-conditioned room (24°C) prior to the test.

QST

Thresholds for cold and warm sensations and for cold and heat pain were assessed quantitatively using the Marstock method of Fruhstorfer *et al.* (1976) (Somedic AB, Stockholm, Sweden).

Microneurography

Microneurography, and identification of different functional types of C fibre were performed as described by Serra *et al.* (1999, 2004). Briefly, a lacquer-insulated tungsten microelectrode, with a shaft diameter of 200 μm and a nominal 1 MΩ impedance (FHC, Bowdoinham, ME, USA) was inserted into the superficial peroneal nerve at the level of the ankle and guided into a sensory fascicle by intraneural microstimulation. The intraneural signal, referred to a second tungsten needle inserted into the skin close by, was amplified (FHC Inc., 3+ Cell Isolated Microamplifier), noise eliminated (Hum Bug, Quest Scientific, North Vancouver, Canada) and digitized at 10 kHz using a PC with QTRAC software (Institute of Neurology, University College London, London, UK). Electrical stimuli were delivered to two needles resting on the surface of the skin in the area on the dorsum of the foot to which the sensations induced by intraneural microstimulation were projected. Stimuli (0.2 ms duration) were delivered every 4 s and multiple C-units were recognized by (usually straight) lines on a raster plot of latencies of peaks in the neurogram.

To identify the functional class of the units, the baseline stimulation rate of 0.25 Hz was interrupted with a 3-minute pause in stimulation and a 3-minute period of stimulation at 2 Hz. Units that slowed progressively by at least 10% during the 2 Hz train were identified as nociceptors (Type 1; Serra *et al.*, 1999). These were further separated into mechano-sensitive and mechano-insensitive nociceptors (Types 1A and 1 B, respectively; Serra *et al.*, 2004) by the 3-minute pause in stimulation, since only the mechano-insensitive nociceptors slow by >2% at 0.25 Hz after the pause.

Skin temperature was measured with a thermocouple taped to the skin, close to the receptive field(s) of the unit(s) under study. It was controlled simply by moving a shielded infra-red lamp closer to, or further away from, the patient, or by switching it off. Although crude, this procedure was preferred to an automatic lamp controller using temperature feedback because it avoided electrical interference due to electronic switching of the lamp current and it did not generate small oscillations in temperature and therefore in C-fibre latency, which would interfere with interpretation of impulse activity.

Results

In spite of their ongoing pain, the five patients volunteered for a total of 11 microneurographic recording sessions (three by L.F., four by M.R., two by W.D., one by R.M. and one by S.T.). The patients' C-fibre recordings exhibited a number of abnormal features, including extensive spontaneous activity in nociceptors in M.R. and abnormally low mechanical nociceptive thresholds in R.M. which are described elsewhere (Ochoa *et al.*, 2005).

Table 1 Properties of C-nociceptor units exhibiting double spikes

Unit no.	Patient	Unit type[a]	Conduction velocity[b]	Double-spikes				Spontaneous activity[f]
				Intervals (ms)	0.25 Hz[c]	2 Hz[d]	Heat activated[e]	
1	L.F.	1A	1.21	9.4	–	+	+	–
2	L.F.	1A	0.61	9.2	–	+	+	–
3	L.F.	1A	0.77	20	–	+	+	–
4	L.F.	1A	0.67	48	–	+		–
5	M.R.	1B	0.67	120	+		+	+
6	M.R.	1B	0.37	43, 48	+			–
7	M.R.	1B	0.57	106	+			–
8	M.R.	1B	0.56	8.5, 16.5, 53	+		+	+
9	W.D.	1A	0.74	21, 35	+	+	+	–
10	W.D.	1A	0.70	25.5	–	+		–
11	W.D.	1B	0.95	18	+	+	+	–
12	R.M.	1A		9.1	–	+	+	–
13	S.T.	1A	0.83	15	+	+	+	–

[a]slowing subtype (1A units normally mechano-sensitive, 1B units mechano-insensitive); [b]apparent conduction velocity of fastest branch at 0.25 Hz; [c]+ indicates double spikes recorded at 0.25 Hz, – indicates no double spikes recorded at 2 Hz; [d]+ indicates double spikes recorded at 2 Hz, blank indicates 2 Hz not tested or result not discernible; [e]+ indicates double spikes promoted by warming the skin (no higher than 37°C), blank indicates temperature sensitivity not tested; [f]+ indicates evidence for spontaneous discharges in the unit, – indicates no such evidence.

Here, we will focus on the most common abnormality, the occurrence of double spikes, which were seen in each patient and in nine out of the 11 recording sessions. The 13 units that produced double spikes for a single brief electrical stimulus are listed in Table 1. In three units, double firing occurred at multiple intervals, but extra spikes were never seen at more than one interval in the same sweep. Double firing was most often noticed during repetitive stimulation at 2 Hz because there were abnormal 'kinks' (i.e. abrupt changes of slope) in the profiles of activity-dependent slowing in the latency raster plots. The change in slope indicated a change in the number of impulses being propagated along at least part of the axons, although the stimulation rate was constant. In seven of the units, however, double firing was observed at the baseline stimulation rate of 0.25 Hz; it was noticed because of fluctuations in the baseline latency. Temperature-sensitivity was best revealed by altering the skin temperature of units that showed double spikes at 0.25 Hz, but could be inferred in some additional units by carrying out repetitive stimulation at two temperatures. Examples of these different features of double firing are given below.

Double spikes in C-nociceptors provoked by stimulation at 2 Hz

The first case we observed of impulse multiplication was in patient L.F. (Fig. 1) during repetitive stimulation at 2 Hz, a protocol used to help classify the functional type of C-fibres (Serra *et al.*, 1999). During the first minute of stimulation at 2 Hz, the response alternated between three latencies (Fig. 1A). This 'flip-flop' (Weidner *et al.*, 2003) or 'jumping' (Serra *et al.*, 1999) phenomenon, attributed to impulses in different branches reaching the stem axon first, is normal (except that jumping normally only occurs to longer latencies when latency is increasing). Since one impulse invades only each branch and the stem axon, the jumping does not interfere with the smooth trajectories of activity-dependent slowing in the separate paths. Near the end of the 2 Hz train, however, what looked at first like another 'jump' gave rise to an increased rate of activity-dependent slowing. This phenomenon was also differentiated from the earlier jumps by the fact that impulses at two latencies were recorded during the same sweep (Fig. 1B). Clearly, the increased rate of activity-dependent slowing occurs because at least part of the axon conducts impulses at 4 Hz rather than 2 Hz. The mechanism of generation of the extra impulses is likely to be unidirectional conduction block at a branch point, as previously argued by Weidner *et al.* (2003). For the double impulse to be recorded, the first impulse to reach the branch point must propagate orthodromically but fail to propagate antidromically down the slower branch. The impulse excited in the slower branch is then not destroyed by collision and is able to propagate towards the CNS (see Fig. 2 of Weidner *et al.*, 2004).

Three further examples of double spikes provoked by stimulation at 2 Hz are shown in Fig. 2. Two were additional units recorded from the same patient L.F., while the third was recorded from patient S.T. The example in Fig. 2A (enlarged in Fig. 2B) shows more clearly, on a sweep-by-sweep basis, the relationship between the occurrence of a second spike and an additional increase in latency. In Fig. 2C, the extra spikes occurred during most of the period of 2 Hz stimulation and, in all these cases, they ceased at the end. The association of impulse multiplication with repetitive stimulation—when the axons are relatively hyperpolarized and with higher thresholds—is consistent with it being caused by conduction block at a site of reduced safety factor (see Discussion).

Double spikes with stimulation at 0.25 Hz and effects of changing temperature

Figure 3A shows sections of a recording from a Type 1B nociceptor in W.D. The skin temperature was raised to 36.3°C using an infra-red lamp, when small fluctuations in the baseline recording at 0.25 Hz (amplified in Fig. 3B) suggested spontaneous activity. However, close inspection of the raster plot revealed that each small increase in latency was preceded by a second spike to the previous stimulus at an interval of ~18 ms (Fig. 3B). It is notable in Fig. 3B that the second spike undergoes much smaller latency fluctuations than the first spike. This is consistent with the finding of Weidner *et al.* (2003) that activity-dependent slowing of second spikes was generally less than that of first spikes, and can be explained by the strong effect of activity-dependent hyperpolarization on the velocity recovery cycle: hyperpolarization increases supernormality and therefore causes a relative acceleration of a second spike propagating during the supernormal phase (see, for example, Fig. 6 in Bostock *et al.*, 2003). (However, second spikes at short intervals, close to the entrainment interval of ~10 ms do not show relative acceleration, as was the case with the units in Figs 1, 2C and 2D.)

For the unit in Fig. 3A, repetitive stimulation at 2 Hz did not increase the amount of impulse multiplication, although a kink in the latency trajectory was caused by a few double spikes after ~40 s. They caused a transient increase in slope, which made the latency profile appear to have a sharp bend, in contrast to the normal rounded shape. The lamp was then turned off to lower the skin temperature and, by the time it had reached 33.3°C, the double impulses had ceased, the baseline latency was flat and repetitive stimulation at 2 Hz produced a normal smooth latency trajectory (Fig. 3A, *right*). Apart from the small kink at 40 s, the profiles of activity-dependent slowing at the two temperatures were similar to those in a comparable unit recorded from a normal control subject (Fig. 3C).

In patient S.T., we recorded another mechano-sensitive nociceptor that showed no evidence of impulse multiplication until the skin temperature was raised close to 36°C. After repetitive stimulation at 2 Hz (Fig. 2D), it started giving repeated double spikes during baseline stimulation at 0.25 Hz until the lamp was turned off (Fig. 4). Turning the lamp on again restarted the double spikes.

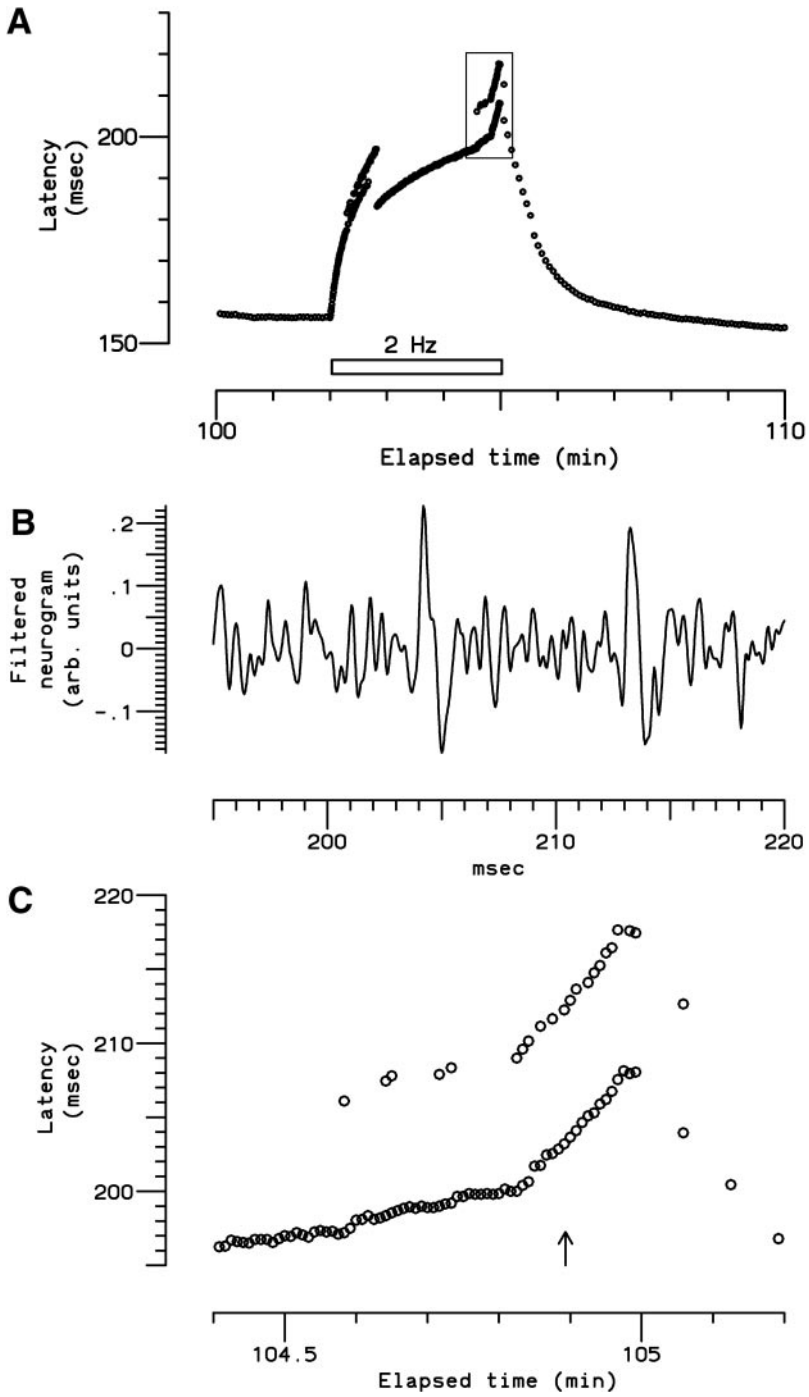

Fig. 1 Double spikes induced by repetitive stimulation at 2 Hz in mechano-sensitive nociceptor of patient L.F. (**A**) Raster plot of latencies at which action potentials were recorded during electrical stimulation. Each dot represents latency of peak of action potential. Baseline stimulation at 0.25 Hz: bar indicates period of stimulation at 2 Hz. Box at 105 min indicates area enlarged in **C**, when double spikes were recorded. (**B**) Nerve signal at time arrowed in **C**, indicating that two spikes occurred during the same sweep. (**C**) Part of raster plot in **A** enlarged to show that presence of second spike coincided with increased slope of activity-dependent slowing.

Comparison of double spike frequency in patients and normal controls

Not all units in the patients exhibited double spikes and double spikes have been observed occasionally in normal subjects (Weidner *et al.*, 2003; our unpublished observations). Moreover, since the skin in the patients was in most cases abnormally warm and we have shown that warming can precipitate double spiking, the question arose as to whether the double spikes in the patients were a consequence of axonal pathology or merely of their increased skin temperature. We estimated that double spikes occurred in about half the

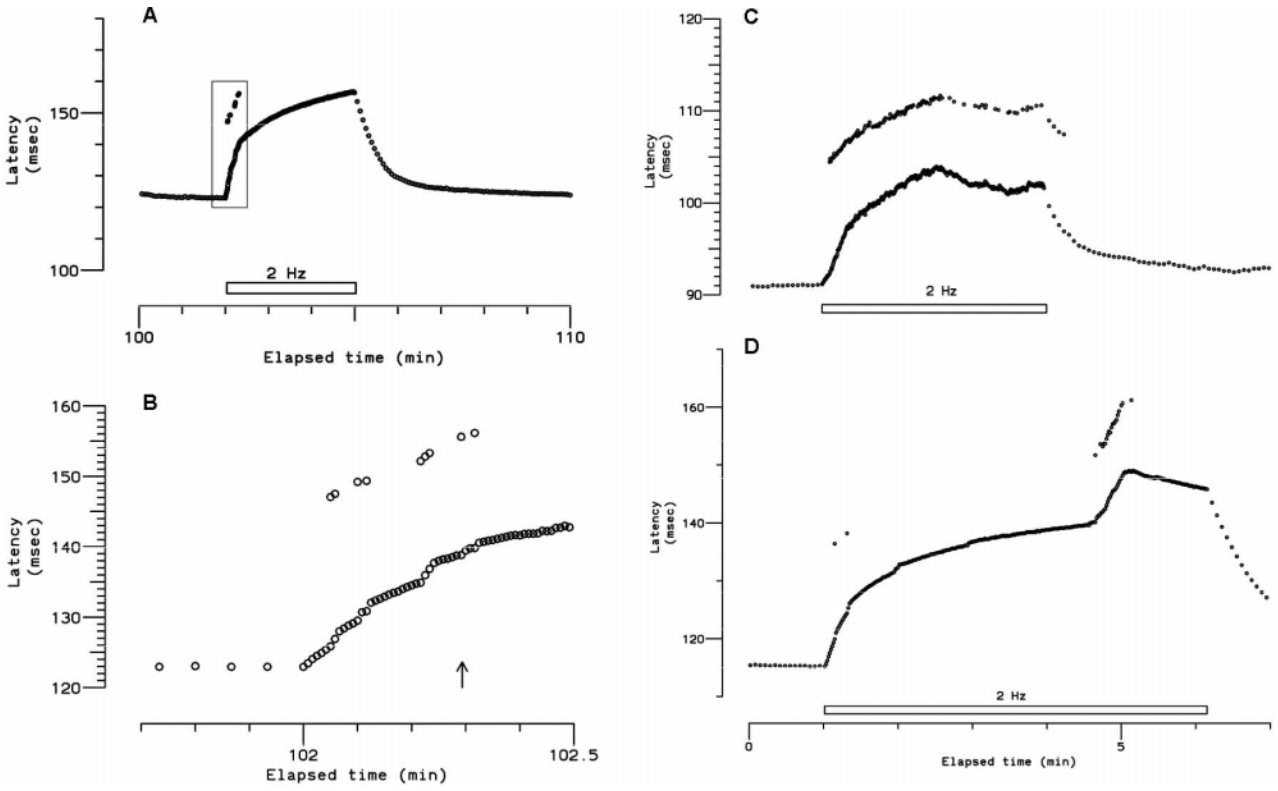

Fig. 2 Further examples of double spikes induced by repetitive stimulation. (**A**) Brief period of double spiking in second mechano-sensitive nociceptor of patient L.F. Area in box is enlarged in **B** to show one-to-one correspondence between presence of second spike in one trace and abnormal increase in latency in the following trace. (**C**) Third double spiking unit recorded from L.F., which gave double spikes almost continuously during period of stimulation at 2 Hz. Partial recovery of latency during the middle of the 2 Hz train corresponds to period of intermittent double spikes. (**D**) Mechano-sensitive nociceptor from patient S.T. showing period of double spikes after repetitive stimulation at 2 Hz for nearly 4 m, which causes increased slope of activity-dependent slowing (as in Fig. 1). Two double spikes also occurred earlier in the train.

nociceptors in the patients; from the 11 recording sessions, we counted 13 units that definitely gave double spikes (because a second spike, indistinguishable in size and shape from the first, was consistently associated with abnormal extra slowing of the first spike) and 14 that definitely did not (because they had a stable baseline latency at 0.25 Hz and smooth profile of activity-dependent slowing at 2 Hz). We excluded from these counts other units that had abnormal profiles of activity-dependent slowing at 2 Hz or irregular baseline latency at 0.25 Hz, when we were unable to distinguish for certain whether these irregularities were due to double-spiking, spontaneous firing or intermittent blocking. The lowest temperatures at which the double spikes occurred were 31.5–37.3°C (mean 34.1°C).

For comparison, we have searched through our recordings from normal subjects made during other studies (Serra *et al.*, 1999, 2004; Bostock *et al.*, 2003; Campero *et al.*, 2004) for nociceptor units that have been stimulated at 2 Hz for 3 min at skin temperatures of 34°C or above. (The majority of recordings were made at lower skin temperatures.) Using the same criteria as for the patients, we found 43 such units that either definitely did or definitely did not give double spikes. These were obtained in 12 recording sessions from nine different subjects, aged 20–58 years. The skin temperatures ranged from 34 to 38.2°C (mean 35.9°C). Of these 43 units, 27 were classified as Type 1A (mechano-sensitive) and 11 as Type 1B (mechano-insensitive), while five could only be classified as Type 1 (nociceptor). Just one of these units, a Type 1A recorded at 36.4°C, exhibited double spiking. The frequency of one in 43 (2.3%), is consistent with the figure of eight in 250 (3.2%) given by Weidner *et al.* (2003) (temperature unspecified), but much lower than the 13 in 27 (48%) found in the patients with neuropathic pain. Although two of the patients were older than any of the controls, there was no evidence that double spiking was associated with old age: the youngest patient at 14 years had a high proportion of units giving double spikes and the only normal subject with a double-spiking unit was, at 24 years, one of the youngest.

Discussion

This study has shown that double spikes occur unusually frequently in the nociceptors of patients with neuropathic pain and hyperalgesia, and that this phenomenon is promoted by warming the skin. Here, we discuss the likely mechanism of generation of the extra impulses, why they should depend on temperature, and whether they may make an appreciable contribution to hyperalgesia.

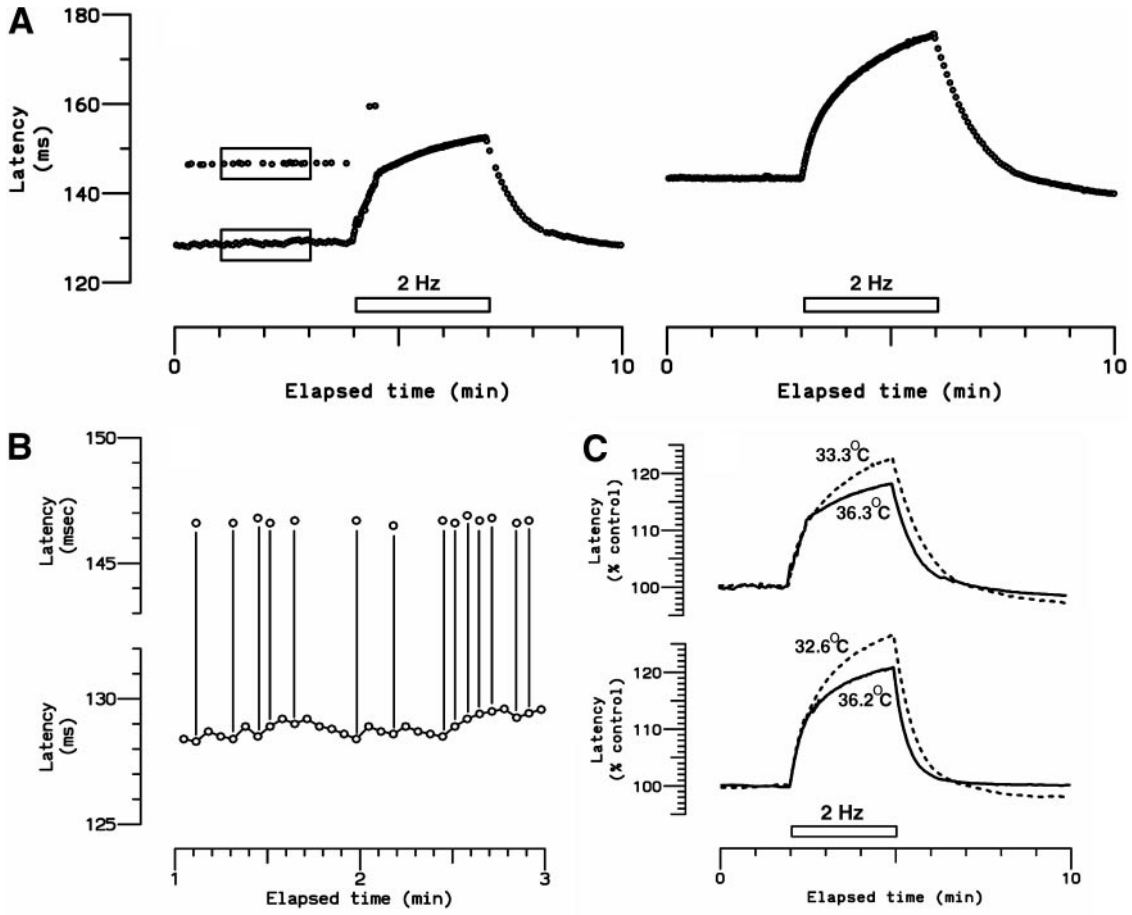

Fig. 3 (**A**) *Left:* Intermittent double spikes recorded during 0.25 Hz stimulation from mechano-insensitive nociceptor in patient W.D. Baseline latency at 36.3°C fluctuated and second spikes were recorded ~18 ms later. A pair of extra spikes during stimulation at 2 Hz caused kink in latency trajectory during 2 Hz stimulation. *Right:* Recording from same unit after reducing the temperature to 33.3°C. Baseline latency was flat, second spikes were absent, and trajectory of activity-dependent slowing during stimulation at 2 Hz was smooth. (**B**) Enlargement of areas boxed in **A** showing latency fluctuations more clearly, a one-to-one correspondence between presence of a second spike and an increased latency to the following stimulus. (**C**) *Top:* Latency trajectories in **A** superimposed and compared with those at similar temperatures from normal subject (Bottom), showing that temperature effects are similar apart from kink due to extra spikes in patient.

Mechanism of extra spikes in C-nociceptors

Double impulses occurring occasionally in normal subjects were attributed by Weidner *et al.* (2003) to unidirectional conduction block at branch points. This hypothesis elegantly accounts for all the features of the double impulses in the patients in this study and provides the most likely explanation. The fact that the second spike has an almost identical waveform to the first indicates that it is propagating in the same axon, as does the increase in activity-dependent slowing. A second spike in the same axon could, in principle, be caused by mechanisms other than unidirectional conduction block. The stimulus could set up a sufficiently strong membrane potential oscillation to generate a second spike at the same site, or another site on the axon might be a hyperexcitable trigger zone, which generates a second spike. The triggering of bursts of action potentials by a single impulse passing a focus of hyperexcitability has been described in normal human motor axons after release of ischaemia (Kugelberg, 1946) or after repetitive stimulation (Bostock and Bergmans, 1994). It is a common experience that some sensory fibres also produce triggered discharges during the post-ischaemic state, but such ectopic discharges seldom stop at a single added impulse. A similar mechanism may operate in polyneuropathies, in which a single stimulus has been found to evoke a short burst of impulses in myelinated afferents (Campero *et al.*, 1998).

In neuromuscular diseases, on the other hand, single extra motor unit discharges are often seen in the EMG (Partanen, 1978) due to ectopic impulses generated distally at or near the junction of myelinated and unmyelinated parts of the terminal motor axon (Roth, 1980). These double discharges usually occur at intervals within the range of 5–7 ms (Roth, 1980), corresponding to the time of peak superexcitability of human motor axons (Kiernan *et al.*, 1996). Peak superexcitability in human unmyelinated axons occurs rather later (Bostock *et al.*, 2003) and at times that overlap with

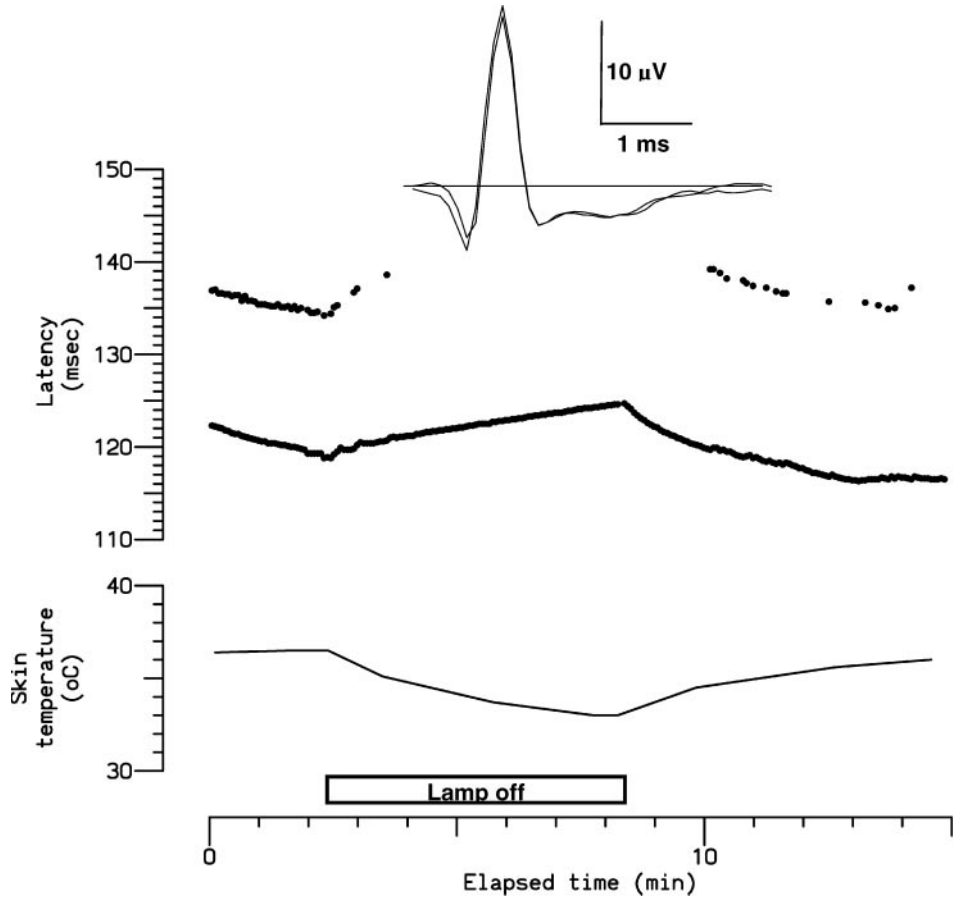

Fig. 4 Effects of temperature on double spikes in mechano-sensitive nociceptor of patient S.T. Bar indicates period when radiant heat lamp was turned off and skin temperature fell from 36.5 to 33°C. Double spikes were inhibited by cooling the skin and restarted when it was warmed up again. The effects of the double spikes on activity-dependent slowing are masked by the effects of the changes in temperature. *Inset*: superimposed waveforms (inverted) of 1st and 2nd spikes, averaged over the 15-minute period (same unit as in Fig. 2D).

the interspike intervals in this study. Thus, a pathological re-excitation of C-nociceptors at the time of peak superexcitability, analogous to the double discharges in the EMG, seems plausible. However, re-excitation at a trigger zone would be expected to send impulses in both directions to propagate along the full course of the axon between stimulating and recording sites, as occurs in the case of the double discharge described by Roth (1980). In that case, the activity-dependent slowing due to the second spike should be the same as that caused by an extra electrical stimulus. We have obtained no relevant data from the patients, but Weidner *et al.* (2003) reported that this is not the case for double impulses recorded in normal subjects. Their second spikes caused consistently less activity-dependent slowing than an extra electrical stimulus, indicating that part of the axon escaped the second spike, consistent with the unidirectional block hypothesis. Extra impulses might also arise by reflection at branch points, since these are electrically analogous to sites of demyelination in myelinated axons, which have been found to be a source of impulse multiplication (Howe *et al.*, 1976; Calvin, 1982). The extra membrane capacitance at such sites delays repolarization, such that the action potential can outlast the refractory period of a neighbouring stretch of excitable membrane and cause re-excitation. However, reflections at branch points would also be expected to cause as much activity-dependent slowing as an extra stimulus and therefore also seem less likely than unidirectional conduction block as the source of impulse multiplication in C-nociceptors.

Why is impulse multiplication provoked by repetitive stimulation?

The electrical analogy between axonal branch points and sites of demyelination in myelinated fibres, referred to above, helps to explain why unidirectional conduction block should be provoked by repetitive stimulation. Conduction block in demyelinated axons is often frequency related, and this is a likely cause of fatigue in demyelinating disease (Waxman, 1988; Kaji *et al.*, 2000). It has been shown that activity-dependent conduction block in single demyelinated A fibres is due to the rise in threshold and, therefore, the reduction in safety factor caused by membrane hyperpolarization by the electrogenic sodium pump (Bostock and Grafe, 1985).

C-fibres experience much greater changes in intracellular sodium concentration per impulse and exhibit more pronounced activity-dependent hyperpolarization such that, at branch points where the safety factor is low, a critical level may occur at relatively low rates of repetitive stimulation. However, the relationship between repetitive stimulation and safety factor was not simple: unidirectional conduction block often increased only temporarily during the 2 Hz train.

Why is impulse multiplication temperature dependent?

The analogy between axonal branch points and sites of demyelination also helps to explain why unidirectional conduction block should increase on warming the skin. It is well known that symptoms of demyelination in multiple sclerosis can be brought on by mild infections, exercise, a hot bath or anything else that raises core temperature (Guthrie, 1951; Nelson and McDowell, 1959). Temperature-dependent conduction block in single demyelinated fibres was recorded by Rasminsky (1973) and explained by the fact that warming speeds sodium channel inactivation, shortens the action potential, and therefore reduces the time available to depolarize the extra membrane capacitance of a demyelinated axon (Koles and Rasminsky (1972). Similar considerations are expected to apply at an axonal branch point and, indeed, this has been found experimentally and modelled for branch points in the squid axon (Westerfield *et al.*, 1978).

Why should conduction failure be unidirectional?

Grossman *et al.* (1979*a*, *b*) recorded differential failure of conduction into two branches of an umyelinated lobster axon during repetitive stimulation. Modelling by Parnas and Segev (1979) indicated that axonal diameter was not an important factor and, experimentally, the block occurred in both branches at the same time after blocking the electrogenic Na^+/K^+ pump. Differences in specific membrane properties, rather than axonal diameter are therefore likely to underlie the unidirectional conduction failure in C-nociceptors. For example, differences that would make conduction block less likely for an impulse propagating into the proximal arm of a branch, compared with one propagating into a distal branch, would be a higher density of sodium channels proximally or of potassium channels distally. We can only speculate that such changes might come about in painful neuropathies through one of the many pathological changes that have been reported in axonal transport in nerve disease (Ochs and Brimijoin, 1993). In animal models of inflammatory and neuropathic pain states, differential up-regulation and down-regulation of several types of sodium channel is thought to contribute to the pathophysiology (Baker and Wood, 2001). A small accumulation of sodium channels being transported distally at the proximal side of branch points would be sufficient to bias conduction failure at these sites of reduced safety factor in the appropriate direction.

Do the double spikes make an appreciable contribution to hyperalgesia?

It is not possible from our present data to quantify the likely contribution to sensation of the impulse-doubling phenomenon we observed. We can only outline a few relevant considerations. In our experiments, axons are excited in a very small area of skin surrounding a needle resting on the surface and by single brief stimuli. Natural stimuli, in contrast, are likely to activate receptors in a larger area of skin and to evoke bursts of impulses from each. The number of collisions between impulses arising in different branches may, therefore, be much greater for natural stimuli and hence the potential for unidirectional conduction block to affect the number of impulses propagating centrally may also be greater. Of course, the unidirectional block would have to have the correct directionality because, without a bias in favour of conduction at branch points being blocked in the antidromic direction (as discussed above), there would be no net increase in impulse propagation to the CNS. An important factor is that impulse multiplication increases the frequency of impulses reaching the CNS as well as the number of impulses. This effect is perhaps most striking for the experimental electrical stimulation in this study, where the instantaneous impulse rate jumped from no more than 2 Hz to as high as 100 Hz. Subjectively, two electrical stimuli 10 ms apart are much more painful than the same two stimuli 500 ms apart [an observation volunteered by many subjects in a previous study on recovery cycles (Bostock *et al.*, 2003)], although we are not aware that this effect has been quantified rigorously in humans. With natural stimuli generating bursts of impulses, the effects on instantaneous firing rates may be less striking, but clearly there will be an increase in pain sensation.

In conclusion, we have found a relatively high incidence of double spikes attributable to unidirectional conduction block, in C-nociceptor fibres of patients with neuropathic pain and hyperalgesia. This phenomenon is expected to increase the pain sensations evoked by suprathreshold stimuli, i.e. to contribute to hyperalgesia. Moreover, the impulse doubling was affected by skin temperature appropriately to account for the exacerbation of hyperalgesia by warming, which was a common finding in these patients. Unidirectional conduction block, therefore, makes a previously unrecognized peripheral contribution to this symptom provided that the directionality is biased appropriately.

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