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RESEARCH ARTICLE

An investigation into the peripheral substrates involved in the tactile modulation of cutaneous pain with emphasis on the C-tactile fibres

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Abstract We recently demonstrated the emergence of touch-evoked pain (allodynia) during innocuous tactile stimulation of the skin overlying a painful muscle. This effect appeared to depend on a class of low-threshold unmyelinated mechanoafferents, termed C-tactile fibres (CT). In this study, we investigated the peripheral neurocircuitry of allodynia when pain originates in the skin. Psychophysical observations were carried out in 28 healthy subjects. Cutaneous pain was induced by infusing hypertonic saline (HS: 5 %) into the hairy skin overlying tibialis anterior muscle. An innocuous tactile stimulus (sinusoidal vibration: 200 Hz-200 μm) was concurrently applied to the hairy skin ~90 mm distal to the HS-infusion site. The contribution of different fibre classes to allodynia was determined by employing conduction blocks of myelinated (sciatic nerve compression) and unmyelinated (intradermal anaesthesia, Xylocaine 0.25 %) fibres. In absence of background nociceptive input, vibration was reported as non-painful. During cutaneous pain, vibration evoked a significant and reproducible increase in the overall pain intensity (allodynia). The blockade of myelinated fibres abolished the vibration sense, but the vibration-evoked allodynia persisted. Conversely, the blockade of unmyelinated cutaneous fibres abolished the allodynia (while the myelinated fibres were conducting or not). On the basis of these findings, in addition to our earlier work, we conclude that the allodynic effect of CT-fibre activation is not limited to nociceptive input arising from the muscle, but can be equally realized when pain originates in the skin. These results denote a broader role of CTs in pain modulation.

Keywords C fibre · Cutaneous pain · Allodynia · Hypertonic saline · Vibration · Intradermal anaesthesia

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Introduction

It is widely appreciated that tactile and pain sensations rely on limited classes of peripheral afferent nerve fibres and specialized receptors. However, it is unclear whether these distinct sensations result from the activation of a single class of afferent fibre or the convergence of inputs arising from multiple classes. Resolving this lack of clarity may be clinically advantageous, particularly in conditions where the distinction between touch and pain is blurred such that an otherwise innocuous tactile stimulus is perceived as painful, that is, allodynia (Merskey and Bogduk 1994). Furthermore, in many pain states, it is not possible to resolve whether the observed allodynia reflects an alteration in the peripheral responsiveness of nociceptive afferents or a perturbed central integration of nociceptive and tactile inputs.

The generation of tactile allodynia during experimentally induced as well as pathological pain states is often linked to the activation of low-threshold large-diameter (Aβ) mechanoreceptors (Campbell et al. 1988; Maihöfner et al. 2003; Treede and Cole 1993; Wasner et al. 1999). This attribution is reinforced by observations that electrical intraneural microstimulation of large mechanosensitive units in the context of pain evoked allodynia, whereas the use of large-fibre conduction blocks (compression or ischaemic) abolished this effect (Cervero and Laird 1996;

Gracely et al. 1992; Landerholm and Hansson 2011; Torebjörk et al. 1992). Vibration—a stimulus that can be generated reproducibly using precisely defined parameters—is routinely used in the clinical setting to assess the functional integrity of Aβ mechanoreceptors (e.g. Dellon 1980). However, in the context of pain, although some studies have reported variable, if not opposing, frequency and regionally dependent effects of vibration, few, if any, have tested whether the observed effects are attributable to large- or small-diameter fibres (e.g. Ayles et al. 2011; Fillingim et al. 1998). Furthermore, the varying efficacy of pain relief techniques (e.g. transcutaneous electrical nerve stimulation) that hinge on the 'Gate Control theory' (i.e. large-diameter mediated inhibition of nociceptive processing: Melzack and Wall 1965) is noteworthy; although temporary pain relief has been observed by large-fibre stimulation, there have been reports of exacerbated pain conditions (Melzack 1975; Richardson et al. 1981). In contrast to large-fibre-mediated effects, other studies involving patients with pain-producing conditions have argued for a role of small-diameter 'nociceptive' afferents (Cline et al. 1989; Price et al. 1992). What lent credence to this finding was the abolition of allodynia following blockade of small-diameter *presumed nociceptors* in patients with ongoing pain (Arner et al. 1990; Gracely et al. 1992; Koltzenburg et al. 1994) and the persistence of allodynia following blockade of large-diameter fibres (Cline et al. 1989).

The existence of C low-threshold mechanoreceptors (CLTMRs) was documented long ago in the hairy skin of non-human primates and subprimates (Bessou et al. 1971; Douglas and Ritchie 1957; Maruhashi et al. 1952; Zotterman 1939). Although initially regarded as an evolutionary vestige (Kumazawa and Perl 1977), recent microneurography studies have unequivocally shown the existence of a class of unmyelinated mechanosensitive afferents in human hairy skin (Johansson et al. 1988; Nordin 1990; Vallbo et al. 1993). Akin to CLTMRs, this afferent class, termed C-tactile fibres (CT), can be activated by innocuous as well as noxious mechanical stimuli (Seal et al. 2009; Vallbo et al. 1999). However, whether CTs are the exact human homologue of CLTMRs remains to be ascertained. Notably, our understanding of the CT-fibre functionality is limited to the use of slowly moving, low-force stimuli, for example, soft brushing, where parallel shifts in CT-fibre discharge and affective rating were taken to suggest a role of this afferent class in subserving the perception of pleasantness (Löken et al. 2009). However, it should be noted that brush-stroking can also evoke a neutral or unpleasant sensation at the lowest brushing velocities, suggesting that gentle tactile stimulation can elicit positive and negative affective dimensions of touch (Löken et al. 2009; Löken et al. 2011).

There is a profound paucity of information on the CT-fibre responsiveness to rapidly moving stimuli such as vibration, let alone whether the vibration-evoked C-fibre discharge correlates with a perceptual response. However, based on limited observations in two patients with large-fibre dysfunction, where the application of vibration revealed an impaired capacity for detection (Olausson et al. 2002, 2008), it has been suggested that the CT-fibre responsiveness is limited to slowly moving stimuli. We recently demonstrated that the concurrent application of vibration during underlying *muscle pain* results in an overall increase in pain intensity (allodynia)—an effect found to be reproducible by applying brushing stimuli at speeds known to excite CTs. More importantly, the blockade of myelinated fibres had no discernible effect on the expression of vibration-evoked allodynia, thereby indicating the role of CTs in mediating allodynia during ongoing deep somatic pain (Nagi et al. 2011). However, it remains untested whether CT-fibre activation produces a similar modulatory effect when pain originates in the skin (Jänig 2011). In this study, we tested the perceptual effect, and peripheral origin, of cutaneous vibration on hypertonic saline-induced pain arising from an adjacent skin region prior to and following the use of differential nerve blocks.

Methods

In order to test whether our earlier observations of CT-mediated allodynia during muscle pain (Nagi et al. 2011) are reproducible in a cutaneous pain state, innocuous tactile and pain-producing stimuli were applied to adjacent regions of skin. A total of 28 healthy human subjects (15 males and 13 females) aged 18–40 years, with no reported musculoskeletal disorders, took part in this study. Informed consent was obtained from each subject. All experiments were approved by the UWS Human Research Ethics Committee (approval number: H9190) and complied with the principles of the Declaration of Helsinki.

Each experiment examined the impact of 200 Hz–200 μm vibration on cutaneous pain induced by injecting hypertonic saline (HS: 5 %) into an adjacent region of skin overlying the tibialis anterior (TA) muscle—the vibration and HS-infusion sites were separated by ~90 mm. In all experiments, subjects sat comfortably on a chair with both legs resting on a bench top. As TA is readily palpable during inversion of the foot and dorsiflexion of the ankle joint (Drake et al. 2005), the subjects were asked to perform these movements in order to exclude any unappreciated or undisclosed tenderness.

Cutaneous vibration

A circular Perspex (Plexiglas) probe with a rounded 4-mm-diameter tip was gently placed against the skin of anterior

leg without compressing the underlying structures. The probe was positioned normal to the skin surface, ~150 mm distal to the tibial tuberosity and ~15 mm lateral to the anterior border of tibia. The probe was attached to a feedback-controlled sinusoidal stimulator similar to that used in earlier studies (Mahns et al. 2006; Nagi et al. 2011). Vibration was presented in duplicates prior to and following the induction of cutaneous pain. Each period of vibration lasted 30 s and was repeated at 45-s intervals. This was aimed at providing sufficient time between trials in order to circumvent desensitization of activated mechanoreceptors—large diameter as well as small diameter (Iggo 1960; Sahai et al. 2006; Wiklund Fernström 2004). The frequency (200 Hz) and amplitude (200 μm) values were carefully chosen in order to generate a clearly innocuous, perceptible stimulus (Mahns et al. 2006; Merzenich and Harrington 1969), but also based on our previous findings that it can evoke CT-mediated allodynia in the skin overlying a painful/sore muscle (Nagi and Mahns 2013; Nagi et al. 2011).

Hypertonic saline-induced cutaneous pain

In 25 subjects, cutaneous pain was induced by inserting a 30 G needle just beneath the skin surface, which was then advanced (5–10 mm) parallel to the skin surface and held in place using microporous adhesive tape: the tip of the cannula was positioned ~60 mm distal to the tibial tuberosity and ~30 mm lateral to the anterior border of tibia. The needle was connected via a scalp vein (non-toxic, non-pyrogenic) to a computer-controlled syringe pump (model 55-2226, Harvard Apparatus, Holliston, MA, USA). The rate of HS-infusion was initially set at 50 μl min^{-1} and the response of the subject closely monitored. In control experiments, which were conducted in separate sessions, 5 subjects were asked to report any perceptual response to an infusion of normal (0.9 %) saline at room temperature (infusion rate: 50 μl min^{-1}; 2-min duration; tested in duplicate at 2-min interval) on a pain scale. In test experiments, once the subject identified the onset of the pain associated with the infusion of HS, which typically came about in ~15–30 s, the infusion rate was adjusted (where needed) in order to maintain a constant pain rating. Once a stable baseline pain was obtained, no further adjustments were made to the infusion rate for the duration of the experiment.

Subjects rated the perceived pain intensity using a rotating dial mounted on a Visual Analogue Scale (VAS). The VAS was divided into ten equal segments within a range of 0 (no pain) to 10 (intolerably intense pain) over 300° of rotation. Using the VAS dial, subjects provided a continuous rating of pain prior to, during and upon cessation of HS-infusion, which was recorded on a computer using a digital-to-analogue converter and interface software (Spike2 version 6.05a, Cambridge Electronic Design, Cambridge, England). Once cutaneous pain was initiated and had remained steady for at least 1 min, the intermittent (30-s on, 45-s off) 200 Hz–200 μm vibration was re-commenced. At least two consistent (and consecutive) allodynic responses to vibration and the return to a stable baseline pain were required before progressing with the experiment. In order to avoid response/expectation bias, the subjects were blinded to this information and were instructed that the HS-induced pain could remain the same, increase or decrease during vibration. White noise was delivered through headphones to ensure that auditory cues associated with mechanical stimulator were not detected by the subjects. Furthermore, this approach is consistent with other psychophysical studies that reported an enhanced vibrotactile sensibility when subjects were tested in presence of white noise (Merzenich and Harrington 1969).

Series 1: Cutaneous local anaesthesia

VAS responses to at least two consecutive vibration trains were recorded in 19 subjects. Thenceforth, in 12 of those subjects, 0.2–0.4 ml of a local anaesthetic (Xylocaine: 0.25 %) was injected into the skin region stimulated by vibration in order to preferentially block the C-fibre inputs. The effectiveness of the C-fibre block was ascertained by the loss of perceptiveness to a warm stimulus (~40 °C, brass rod in contact with the skin for 5-s), whereas the preservation of vibration (20 and 200 Hz; 200 μm; 30-s duration) and cool (~15 °C, brass rod in contact with the skin for 5 s) sensations was taken for the intactness of myelinated fibres (Hallin and Torebjörk 1976; Mackenzie et al. 1975; Torebjörk and Hallin 1973). Vibration was applied within the anaesthetized skin (C fibres blocked) and in the adjacent non-anaesthetized skin (all fibres intact).

Series 2: Compression block of sciatic nerve

In 14 subjects (5 naive), a compression block of sciatic nerve was used with the aim of knocking out the myelinated fibres, thereby examining the contribution of C fibres alone to allodynia. However, a clear blockade could not be achieved in 3 subjects—hence, these experiments were terminated before progressing to the HS-infusion stage (data not shown). A metal bar was placed just distal to ischial tuberosity to apply compression to sciatic nerve. Induction of HS-induced cutaneous pain was timed to coincide with the preferential blockade of large- and small-diameter myelinated afferents. The blockade of myelinated fibres was confirmed by the loss of vibration (20 and 200 Hz; 200 μm) and cool (~15 °C brass rod) sensations. Continuity of the C-fibre inputs was confirmed by the preservation of warm (~40 °C brass rod) sensibility. In addition, the production of cutaneous pain (HS-induced) within the

skin region of absent myelinated fibres was itself an indication of intact C fibres (Mackenzie et al. 1975; Nagi et al. 2011; Ochoa and Torebjörk 1989; Weerakkody et al. 2003). Somatosensory sensibility within the skin region affected by compression block was compared with regions on the medial aspect of the experimental leg (innervated by femoral nerve: Drake et al. 2005), and the contralateral leg. VAS responses to vibration were recorded in the skin region with blocked myelinated fibres. In addition, akin to *Series 1*, a small amount of low-dose anaesthetic (Xylocaine 0.25 %) was injected around the vibration site in order to block C fibres—the only class of fibres intact within the innervation territory of sciatic nerve during compression block. The effectiveness of the C-fibre block was ascertained by the loss of warm sensibility. Vibration was applied within the anaesthetized region (all-cutaneous fibres blocked) as well as in the adjacent non-anaesthetized skin (C fibres intact).

Statistical analyses

Each VAS response to vibration was compared to the baseline HS-induced cutaneous pain (base) observed just prior to vibrotactile stimulation and treated as an independent, sequential event. The responses to vibration were also expressed as a percentage of the baseline cutaneous pain. A one-way repeated measures analysis of variance (ANOVA) was used to determine if there was a significant difference between any of the groups (Zar 1984). Where a significant difference was indicated ($P < 0.05$), specific groups were compared using a Newman–Keuls multiple comparison test. All statistical comparisons were completed using Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). All data are presented as mean ± standard error of the mean (SEM).

Results

In 21 of the 25 subjects where HS was infused, a stable baseline was obtained that persisted for the duration of the experiment. Data from the remaining 4 subjects were discarded because a steady background pain could not be achieved or a vasovagal response presumably elicited during HS-infusion (van Lieshout et al. 1991). HS was not used in those 3 subjects where compression block failed to take effect. In parallel experiments ($n = 5$), the infusion of normal (0.9 %) saline into the skin was reported as non-painful (VAS = 0). Although the relationship between the magnitude of HS-induced pain and vibration-evoked responses is shown in Fig. 1a, this was not the primary

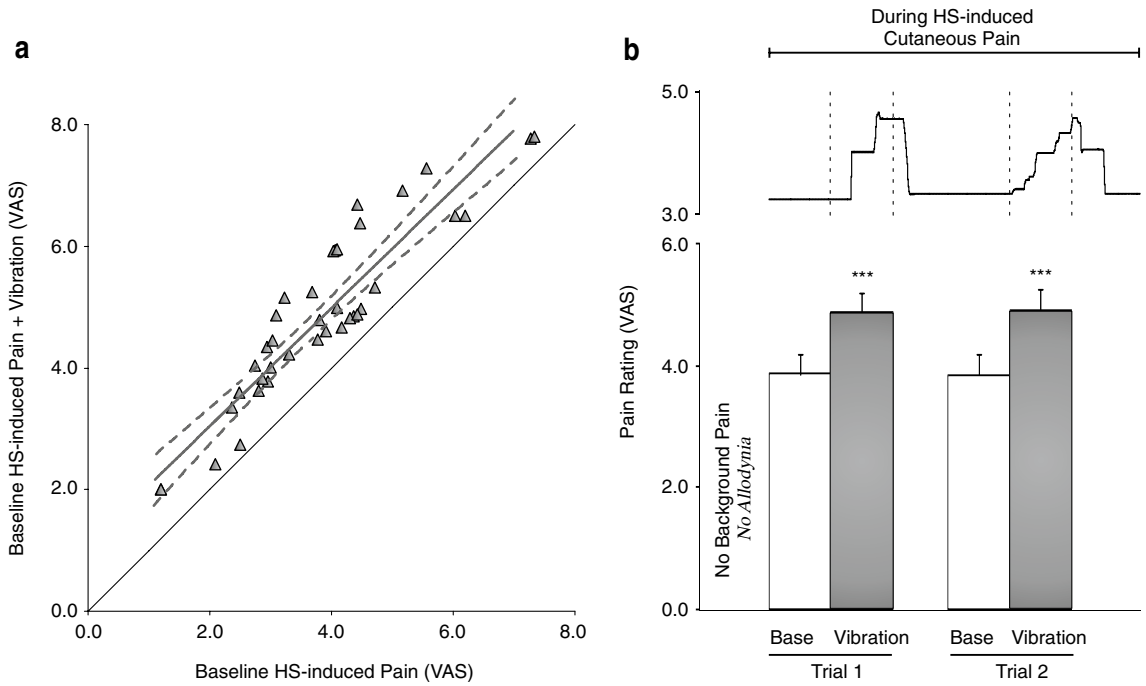

Fig. 1 Reproducible effect of vibration on hypertonic saline-induced cutaneous pain. **a** Duplicate vibration-evoked responses of each subject are *plotted* as a function of the baseline pain (linear regression: *solid grey line*; 95 % confidence intervals: *dashed lines*; $r^2 = 0.85$). Vibration evoked a reproducible increase in baseline pain across all subjects (*triangular points*, above the line of unity). **b** VAS trace of an individual subject and pooled mean pain ratings (±SEM; $n = 19$) prior to (*unshaded bars*) and during cutaneous vibration (*grey bars*) are shown. During HS-infusion, vibration evoked a significant increase in baseline pain. Conversely, in absence of background pain, vibration was reported as non-painful. ***$P < 0.001$

focus of this study. Instead, the aim was to identify the peripheral origin of vibration-evoked allodynia during HS-induced cutaneous pain. Nonetheless, a linear regression analysis ($r^2 = 0.85$) revealed that while the slopes did not significantly differ (0.96 ± 0.07, $P = 0.59$) relative of the line of equivalence ($x = y$ or base = vibration), the regression line was significantly elevated ($x = 0$, 1.18 ± 0.28, $P < 0.0001$), thereby indicating that vibration had a comparable effect across a broad range of HS-induced baseline pain intensities. Prior to the induction of cutaneous pain, all subjects reported that the application of vibration to the skin overlying TA was non-painful. Vibratory sensations were described as a combination of localized pressure at the vibration site, and diffuse vibration within the leg.

Vibration-evoked allodynia

Following the onset of HS-induced cutaneous pain, a reproducible vibration-evoked increase (allodynia) in the overall pain rating was observed on a trial-by-trial basis in all 19 subjects (Fig. 1a). Consistent with the individual responses, the mean data ($n = 19$; ±SEM; Fig. 1b) revealed a significant and reproducible increase in the intensity of HS-induced cutaneous pain (base: 3.8 ± 0.3, 3.8 ± 0.3) during consecutive periods of vibration (vibration: 4.9 ± 0.3, 4.9 ± 0.3; $P < 0.001$; ANOVA: $F = 47.47$, $P < 0.0001$). This effect was stimulus-locked as the VAS rating invariably returned to the HS-baseline before the onset of subsequent periods of vibration. Furthermore, consistent with the stability of HS-induced pain and the reproducibility of vibration-evoked allodynia, there were no significant differences ($P > 0.05$) between duplicate base or vibration values.

Series 1: Allodynia abolished by blockade of cutaneous C fibres

Low-dose intradermal anaesthetic (0.25 % Xylocaine) was used to block the action of cutaneous C fibres, while the myelinated fibres remained intact, in order to examine the effect of this selective blockade on the generation of allodynia. The selective loss of C-fibre activity at the vibration site was confirmed by the abolition of warm sensibility and the preservation of vibration and cool sensations. As shown in Fig. 2, prior to intradermal anaesthesia, HS-infusion evoked a steady baseline pain (base: 100 %) that increased significantly during vibration (vibration: 129.2 ± 4.8, 129.4 ± 4.6 %; $P < 0.001$; $n = 12$). Following C-fibre cutaneous block, in 6 of the 12 subjects, vibration-evoked allodynia was abolished (vibration = base) and, in the remaining 6 subjects, the magnitude of allodynia was reduced by between ~3 and ~37 % (average 8.1 ± 6.0). Consequently, on average, the allodynia evoked within the anaesthetized region was significantly reduced ($P < 0.001$)

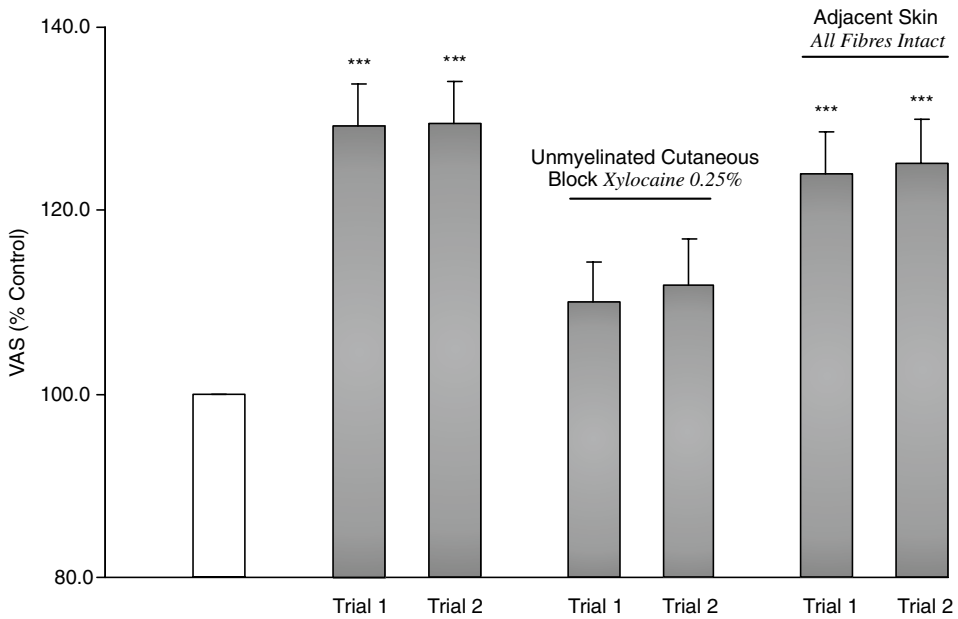

Fig. 2 Allodynia abolished by conduction block of cutaneous C fibres. Mean vibration-evoked responses (±SEM; $n = 12$; *grey bars*) were expressed as a percentage of the baseline (100 %, *unshaded bar*) HS-induced cutaneous pain. Prior to intradermal anaesthesia (i.e. all fibres intact), vibration evoked a significant increase in baseline cutaneous pain (allodynia). Within the anaesthetized skin where the C fibres were blocked (while the myelinated fibres remained intact), the allodynic effect of vibration was significantly attenuated compared to its pre-anaesthetic control ($P < 0.001$) and was statistically indistinguishable from the baseline pain ($P > 0.05$). In the adjacent (non-anaesthetized) skin with all fibres intact, allodynia was preserved. ***$P < 0.001$

relative to the vibration-evoked responses prior to the anaesthesia of C fibres and was statistically indistinguishable ($P > 0.05$) from the baseline HS-induced pain (base: 100 %; vibration: 110.0 ± 4.4, 111.9 ± 5.1 %; $n = 12$). The attenuation/abolition of allodynia by the anaesthesia of C fibres in the skin was apparently not due to a decline in the capacity of peripheral nerve fibres to mediate this effect and nor an inability of the central nervous system to integrate/process the information, as the vibration-evoked allodynia was preserved in the adjacent non-anaesthetized skin (base: 100 %; 124.0 ± 4.7, 125.0 ± 4.9 %; $P < 0.001$; $n = 12$). No significant differences ($P > 0.05$) were found in the magnitude of allodynia within each specific region (i.e. intact, C-fibre blocked and adjacent/non-anaesthetized). That the allodynia was reproducibly evoked while all fibres were intact, but was all but abolished when C-fibre input was blocked, demonstrates the role of cutaneous C fibres in mediating this effect (ANOVA: $F = 18.88$, $P < 0.0001$).

Series 2: Allodynia persists during blockade of myelinated fibres

A compression block of the sciatic nerve was employed in order to thwart the action of myelinated fibres and assess the role of the residual (C-fibre) input in mediating allodynia. The preferential blockade of myelinated fibres was confirmed by the abolition the vibration and cool sensations, whereas the intactness of C fibres was confirmed by the preservation of warm sensibility. Amongst the subjects who took part in both sets of experiments, 2 of them reported (without prompting) a change in the quality of baseline HS-pain when all fibres were intact vis-à-vis blockade of myelinated fibres. In case of the latter, the sharp-stinging attribute disappeared (consistent with loss of Aδ fibres), while the aching-burning component remained preserved. In contrast to the significantly attenuated responses observed during selective C-fibre block while the myelinated fibres were intact (*Series 1*), the vibration-evoked allodynia observed following the blockade of myelinated fibres (base: 100 %; vibration: 127.4 ± 6.0, 127.8 ± 7.4 %; $P < 0.001$; $n = 11$) was abolished (base = vibration) in 9 of the 11 subjects (and within a 5 % variance in the remaining 2 subjects) by subsequent blockade (0.25 % Xylocaine) of the residual cutaneous (C-fibre) input (base: 100 %; vibration: 103.7 ± 2.5, 103.7 ± 2.5 %, $n = 11$; $P > 0.05$). The abolition of allodynia was not due to a generalized decline in the responsiveness of peripheral or central neurons as the effect was preserved in the adjacent non-anaesthetized skin (base: 100 %; 130 ± 7.3; 131.5 ± 8.6 %; $P < 0.001$; $n = 11$). No significant differences ($P > 0.05$) were found in the magnitude of allodynia within each specific region (i.e. C-fibre intact, all-cutaneous-input blocked and adjacent/non-anaesthetized with C-fibre input preserved). Given that allodynia was elicited regardless of the blockade of myelinated fibres, but was abolished in the absence of cutaneous C fibres, attests to the capacity of the latter to modulate cutaneous pain (ANOVA: $F = 12.50$, $P < 0.0001$; Fig. 3).

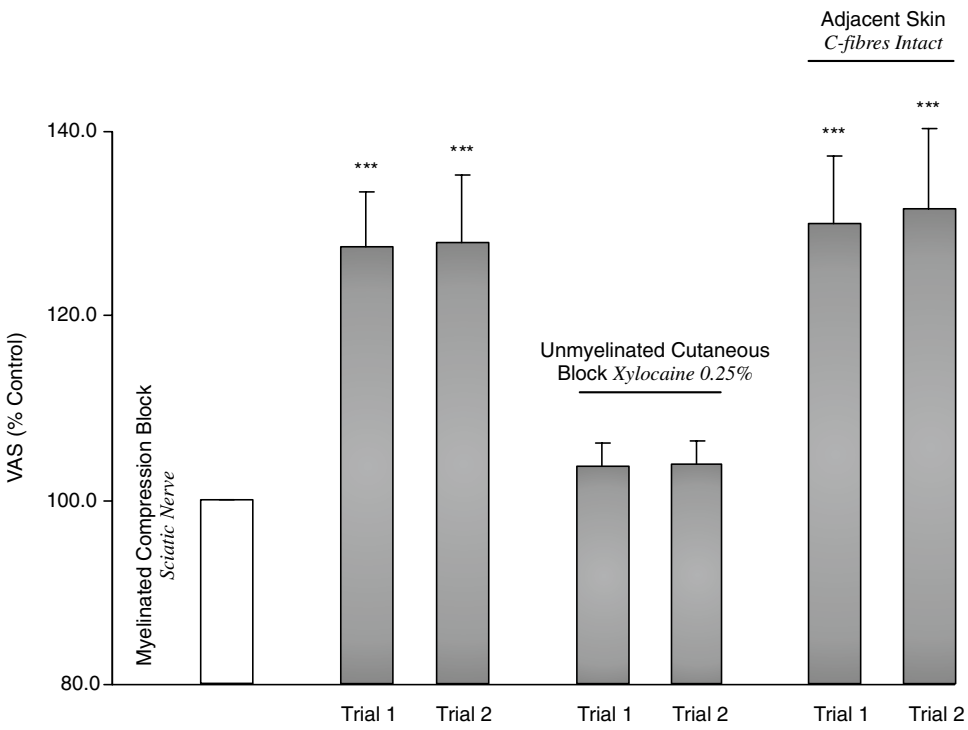

Fig. 3 Allodynia persisted during conduction block of myelinated fibres. Mean vibration-evoked responses (±SEM; $n = 11$; *grey bars*) were expressed as a percentage of the baseline (100 %, *unshaded bar*) cutaneous pain. Vibration evoked a significant increase in baseline cutaneous pain (allodynia) regardless of the blockade of myelinated fibres. In contrast, allodynia was entirely abolished by the blockade of residual (C-fibre) input from the skin region with absent myelinated fibres. In the adjacent (non-anaesthetized) skin with C fibres intact, the allodynic effect of vibration was preserved. ***$P < 0.001$

Discussion

The aim of this study was to investigate the peripheral substrates involved in the tactile modulation of cutaneous pain. In these experiments, we used a slow infusion (50 μl min^{-1}) of hypertonic saline to produce localized pain in the skin that usually came about in half a second or less after the start of the infusion. Conversely, in parallel experiments, the use of normal saline failed to generate any discernible sensation during the 2-min infusion period. In absence of background nociceptive activity, all the subjects described cutaneous vibration as non-painful. However, the application of vibration to the skin during a stable, clearly perceptible level of background pain (induced by HS-infusion into a distant skin region) evoked a reproducible increase in the overall pain intensity; compare with a topical capsaicin model of pain (Andrews et al. 1999; Liljencrantz et al. 2013). This allodynic response was preserved following the blockade of myelinated fibres, that is, the subjects reliably reported an increase in pain intensity during vibrotactile stimulation while the vibration sense had been blocked, thereby indicating the sensitivity of CTs to vibrotactile stimuli, in addition to their contribution to pain processing, albeit apparently not to vibrotactile sensibility. The conduct of CTs during vibrotactile stimulation is in a sense analogous to their thermoreceptive trait. That is, they are activated by rapid cooling of the skin (Douglas and Ritchie 1957; Nordin 1990), and yet the resulting discharge has apparently no bearing on thermoperception (e.g. Mackenzie et al. 1975; Yarnitsky and Ochoa 1991). In contrast to the persistence of allodynia during the blockade of myelinated fibres (while the unmyelinated fibres remained intact), the blockade of cutaneous C-fibre inputs alone was sufficient to abolish the allodynia irrespective of whether the myelinated fibres were conducting or not. Consequently, we now provide evidence that our earlier observations (Nagi et al. 2011), wherein CTs were shown to mediate vibration-evoked allodynia during deep (muscle) noxious stimulation, are not limited to cross-compartment interactions but can be evoked within adjacent regions of skin.

Some of the earlier work engenders a potential ambiguity as to whether allodynia is a product of a central change in the integration of tactile and nociceptive inputs or a peripheral change in the responsiveness of nociceptors due to noxious stimulation. This potential ambiguity is largely due to the confinement of both innocuous and noxious stimuli to a single anatomical compartment. We avoided this ambiguity previously by working across two anatomically distinct compartments, that is, applying a noxious stimulus to one compartment (muscle) and a tactile stimulus to a separate compartment (skin). In our earlier study (Nagi et al. 2011), we argued that, in a two-compartment model, any change in the responsiveness, or sensitization, of cutaneous afferent fibres following intramuscular injection of hypertonic saline was highly unlikely. On this occasion, we likewise argue that peripheral sensitization is unlikely to account for the observed allodynia given the separation (~90 mm) between the stimulation sites for vibration and HS-infusion. Furthermore, the spread of HS to the distal site (where vibration was applied) seems unlikely considering the slow infusion rate (50 μl min^{-1}), the localized nature of perceived pain and the rapid abatement of pain following cessation of infusion. At the infusion rates used in this study, the pain did not appear to be due to localized distension at the infusion site given that no sensation, most notably the absence of pain (VAS = 0), was reported when normal saline (0.9 %) was substituted for HS (5 %).

In both sets of HS-induced responses reported here and in our earlier work (Nagi et al. 2011), the reproducible increase in pain intensity during vibration appears to be more consistent with altered responsiveness (sensitization) at the spinal (dorsal horn) or supraspinal level of the nervous system. Thus, sensitization of dorsal horn neurons may result in a shift in their response properties such that those neurons previously insensitive to a given afferent input may become responsive due to a fall in thresholds or an increase in responsiveness or a combination of both (Woolf 1983; Woolf and Salter 2000; Woolf and Thompson 1991). Consequently, central sensitization may manifest as allodynia which involves an integration of nociceptive and tactile modalities (Merskey and Bogduk 1994). Recently, Seal et al. (2009) proposed that VGLUT3-labelled fibres, a presumed marker of CLTMRs, in mice contribute to the expression, but not the induction, of central sensitization. What lent credence to this proposition was the differential character of responses in the setting of inflammation/injury/trauma, namely a marked diminution of mechanical hypersensitivity in the VGLUT3-knockout mice while the responsiveness to heat (expression of heat hypersensitivity) was unchanged. CLTMRs appear to have direct access to the 'pain' pathways, particularly given the acute nature of HS-induced interactions observed in this study, in addition to the capsaicin-induced interactions reported in rodents and in patients with large-fibre dysfunction (Liljencrantz et al. 2013; Seal et al. 2009). This is consistent with findings on a dense concentration of CT-fibre endings in the superficial laminae of dorsal horn (Andrew 2010; Li et al. 2011; Seal et al. 2009). Indeed, central sensitization may well explain the context-specific contribution of CTs to perception, namely the lack of a distinct percept under normal conditions and the production of pain during background nociceptive input. It is tempting to suggest that an enhanced understanding of the mechanisms underlying these contextual boundaries may

allow for the possibility of modulating the affective regard given to a painful stimulus.

As outlined in the Introduction, large-fibre conduction blocks (compression or ischaemic) have been used to argue for (e.g. Koltzenburg et al. 1994; Landerholm and Hansson 2011) and against (e.g. Cline et al. 1989; Price et al. 1992) the participation of large-diameter fibres in allodynia. Indeed, the result presented in this paper need not preclude the involvement of large-diameter fibres in view of the following: when all fibres were intact, the allodynia was greatly attenuated, albeit not entirely abolished, following C-fibre blockade (Fig. 2); whereas the allodynia observed following compression block of myelinated fibres was abolished by C-fibre blockade (Fig. 3), suggesting that both myelinated and CT fibres can contribute to allodynia. The relative contribution of each afferent class may be stimulus- and/or context-dependent. What predisposes or determines the peripheral contribution to an allodynic expression nonetheless remains to be fully elucidated. Following this premise, previous observations in a large-fibre deafferented subject are worthy of note, wherein the application of an otherwise innocuous monofilament (40 mN) to the untreated skin was perceived as sharp following a capsaicin injection (Cole et al. 2006; Treede and Cole 1993). Although this effect was attributed to small-fibre (Aδ and C) nociceptors, it is tempting to suggest that the role of CTs may be important in such interactions, particularly given their capacity to encode punctate stimuli (Cole et al. 2006; Vallbo et al. 1993) and their newly found role in pain processing (Nagi et al. 2011; Seal et al. 2009).

Indeed, the seemingly unequivocal demonstration of large-fibre-mediated allodynia—generated by intraneural microstimulation of large-diameter fibres in a capsaicin model of experimental pain—is subject to complex (and varied) temporal and spatial patterns (Torebjörk et al. 1992). Most notable was the apparent necessity for there to be an overlap between the region of secondary hyperalgesia (evoked by intradermal capsaicin) and the site at which intraneural stimulation evoked a percept of touch in order for there to be a perceptual shift from innocuous to noxious sensation during activation of large-diameter fibres. In contrast, the CT-mediated effect presented in this paper exhibits a much less constrained interaction such that pain at one site rendered tactile stimulation at a remote locus as painful. This broader participation of CTs in the central integration of tactile and nociceptive inputs was supported by our observations in cross-compartment (i.e. skin and muscle) models in the leg (Nagi and Mahns 2013; Nagi et al. 2011). Furthermore, this broader or less restrained integration of tactile and nociceptive information may underpin certain pathological conditions, wherein the spread of pain does not necessarily follow an orderly somatotopic pattern (e.g. Baron 2009; Fillingim et al. 1998).

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