Role of C-tactile fibers in pain modulation: animal and human perspectives
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C-tactile (CT) fibers, a population of unmyelinated (C) fibers that respond particularly well to gentle stroking, are widely believed to subserve affective touch. However, these fibers (termed C low-threshold mechanoreceptors (C-LTMRs) in non-human mammals) have also been proposed to be involved in the modulation of pain. Intriguingly, functional evidence from both human and animal studies indicates that CT/C-LTMR fibers can both contribute to allodynia as well as mediate pain inhibition. In the spinal cord, C-LTMR fibers form glomerular synaptic arrangements, providing input to several populations of interneurons within the nociceptive circuitry. Thus, the CT/C-LTMR system conveys signals that are subject to intricate processing in the spinal cord and is well-situated within spinal sensory pathways to enable the modulation of pain.

Identification of CT-fiber analogs in non-human mammals
Affective touch is phylogenetically old. Indeed, perhaps all mammalian species engage to various degrees in tactile behavior that appears to serve a primarily or purely social function, from allogrooming in primates to flipper rubbing amongst dolphins [5], and non-discriminatory functions for low-threshold mechanosensation may exist also in other vertebrate and even invertebrate species [6,7]. It is thus not surprising that peripheral nerve fibers with properties similar to human CT fibers have been found also in a number of other species. In rodents, a population of low-threshold mechanosensitive C fibers (C-LTMRs, the common term for these fibers in non-human species; N.B. for convenience, from here on the abbreviation C-LTMRs will be used for all species, including the CTs in humans) was found to express the vesicular glutamate transporter, VGluT3 [8]. Tyrosine hydroxylase (TH) was subsequently found to be expressed in a population of C-LTMR that largely overlaps with the VGluT3+ C-LTMRs [9]. Notably, whereas VGluT3 and TH are selective markers for rodent C-LTMRs, the functional roles for these proteins in C-LTMRs are unclear, as these fibers express other vesicular glutamate transporters and do not express any enzymes apart from TH required for catecholamine synthesis [10,11]. Indeed, neither TH nor VGluT3 appears to be expressed in C-LTMRs in the macaque, although other markers are shared between primates and rodents [12]. Importantly, the existence of a macaque C-LTMR population that is analogous to rodent C-LTMRs suggests that a similar population exists in humans and that this population corresponds to CTs. A second, distinct population of C fiber activated by innocuous mechanical stimuli require a somewhat higher force than TH+/VGluT3+ fibers; these fibers, which express MrgprB4 [13,14], have not been studied as extensively as the former and will for space reasons not be considered further here.

Synaptology of C-LTMRs
VGluT3+/TH+ C-LTMR terminals form a plexus in the inner (ventral) part of lamina II in the rodent spinal cord...
dorsal horn. Ultrastructural observations in rat and mouse spinal cord have found that VGluT3+ C-LTMRs form central terminals of synaptic glomeruli, similar to many other primary afferent fibers; the central C-LTMR terminal has a light axoplasm with loosely packed small synaptic vesicles and numerous mitochondria and lacks neurofilaments and dense core vesicles; thus these glomeruli can be classified as so-called type IIa glomeruli [10]. In support of this, TH+ terminals, identified using a genetically encoded peroxidase, form terminals of similar morphology in lamina II in mice [15]. Curiously, other investigators have identified mouse C-LTMR endings as the central terminals of a subset of type Ia glomeruli, with a morphology similar to those of non-peptidergic C-fiber nociceptors [16]. Further studies are clearly needed to resolve this discrepancy. However, regardless of the precise morphology of C-LTMR-associated glomeruli, it is obvious that the glomerular structure of the nerve endings of C-LTMRs in the rodent spinal dorsal horn points to a capacity for complex integration of C-LTMR-mediated signals already at the level of the first synapse. In fact, both dyadic and triadic synaptic arrangements between the central terminal, postsynaptic dendrites, and peripheral inhibitory axons and dendrites are evident in these types of glomeruli. This indicates much more complex modes of integration than would be expected for a diffuse tactile modality solely contributing to social or affective touch and could suggest involvement also in other mechanical somatosensory modalities. Notably, the morphology and organization of C-LTMRs in the spinal cord have so far not been studied in humans or other primates.

**Downstream circuitry of C-LTMRs**

Unlike low-threshold mechanosensitive Aβ fibers, C-LTMRs terminate solely in the spinal dorsal horn and do not provide axonal branches to dorsal column nuclei. Moreover, C-LTMRs do not form monosynaptic connections with lamina I projection neurons that express the neurokinin 1 receptor (NK1R) [10], or with postsynaptic dorsal column neurons in the deep dorsal horn [17]. At this point, it cannot be excluded that spinothalamic or spinobulbar neurons lacking NK1R could be monosynaptic targets of C-LTMRs; however, the apparent lack of an effect on C-LTMR-mediated touch shown after cordotomy of the anterolateral tract suggests a limited contribution of spinothalamic neurons to C-LTMR-mediated signaling, at least in humans [18]. Notably, in the rat some wide dynamic range lamina I spinoparabrachial lamina I neurons respond to slow brushing, likely via polysynaptic C-LTMR input [19]. Thus, the available evidence suggests that C-LTMR-mediated signals reach supraspinal centers principally via interneurons in laminae II and III of the dorsal horn. A number of interneuronal targets of C-LTMRs have been identified (Figure 1). Already in 1979, monosynaptic C-LTMR input to lamina II cells was observed in the cat [20]; some of these had the morphology of vertical cells, which can provide connections to lamina I projection neurons [21]. More recently, C-LTMR connections to neuronal populations identified primarily by molecular markers rather than morphology have been characterized. Excitatory lamina II/III neurons expressing the γ isoform of protein kinase C (PKCγ) receive substantial synaptic input from C-LTMR terminals [10,17], as does a population of inhibitory neurons identified by the expression of retinoid-related orphan receptor β (RORB) [17] and a distinct subpopulation of neurons expressing gastrin-releasing peptide (GRP) [22]. Parvalbumin-expressing neurons are also major targets of C-LTMR terminals; however, there are both excitatory and inhibitory parvalbumin neurons in the dorsal horn, and whether one or the other of these populations is selectively targeted by C-LTMRs remains unclear [10,17]. C-LTMR synapses onto calretinin neurons are also observed but are relatively sparse [10]. Notably, some other interneurons with extensive input from myelinated LTMRs receive little or no C-LTMR input [10,17].

Interestingly, several of the neuron populations putatively targeted by C-LTMRs have been implicated in nociceptive circuits. For instance, C-LTMRs have possible excitatory access to nociceptive lamina I projection neurons via circuits involving both PKCγ neurons and vertical cells [23], while inhibitory parvalbumin neurons are thought to prevent low-threshold mechanoreceptor signals to such projection neurons [24]. GRP neurons targeted by C-LTMRs may also provide negative modulation of nociception, possibly via enkephalineric neurons [22,25].

**Mammalian C-LTMRs in the modulation of pain and nociceptive signaling**

**Pronociceptive role**

The first indication that C-LTMRs could be involved not only in low-threshold mechanosensation but also in pain signaling was the observation that mice deficient in VGluT3 show impaired acute mechanical nociception as well as attenuated mechanical allodynia in inflammatory and neuropathic pain models [8]. Optogenetic activation of VGluT3+ fibers in the skin further suggested involvement in some forms of allodynia [26]. However, it soon became clear that the assessment of C-LTMR function in mice is complicated by VGluT3 and TH expression in a range of other cell populations in somatosensory pathways; both VGluT3 and TH are transiently expressed in DRG neurons other than C-LTMRs during development [27,28], and VGluT3 is transiently expressed in a dorsal horn neuronal population implicated in mechanical hypersensitivity [29]. Moreover, both TH and VGluT3 are expressed by Merkel cells that form low-threshold mechanosensitive complexes with slowly adapting Aβ fibers [27]. Thus, interfering with C-LTMR function solely based on VGluT3 or TH expression could
conceivably affect a wide variety of cells other than C-LTMRs, substantially complicating interpretation.

A more selective approach towards assessing a contribution of C-LTMRs to nociception in the mouse entailed knocking out the T-type Ca\(^{2+}\) channel Ca\(_{\mathrm{v}}\)3.2 in neurons expressing the Na\(^{+}\) channel Na\(_{\mathrm{v}}\)1.8, which is found in nociceptors as well as C-LTMRs. This resulted in acute cold and mechanical nociception deficits and attenuated nociceptive behavior in the formalin assay as well as reduced allodynia after nerve injury [30]. Furthermore, ablation of a population of primary afferent fibers comprising C-LTMRs as well as MrgrpD-expressing nociceptors strongly impaired formalin-induced pain behavior [31]; as MrgrpGD\(^{-}\) fibers do not contribute to such behavior in the formalin assay [32], this impairment was attributed to the loss of C-LTMRs. On the other hand, mice with selective loss of mechanosensitivity in C-LTMRs show modest or no impairment of mechanical pain hypersensitivity in inflammatory and neuropathic pain models, arguing against a pronociceptive role for C-LTMRs [27].

In human psychophysical studies, brushing at intermediate stroking speeds (which generate a robust response in the C-LTMRs) was tested in the presence of tonic muscle pain induced and maintained by a hypertonic-saline infusion [33,34]. Stable baseline pain was first achieved and monitored for several minutes; then brushing was concurrently applied, comprising multiple strokes lasting 30 s, and this resulted in a >25% increase in the overall pain — an effect that was reproducible and stimulus-locked, that is, the pain returned to baseline during the inter-brushing interval. Importantly, this increase in pain to brushing (an example of allodynia) persisted during a preferential conduction block of myelinated fibers by compression. The efficacy of the myelinated block was confirmed by the abolition of vibration and innocuous cold sensations — tests for A\(\beta\)-fiber and A\(\delta\)-fiber function, respectively — while a preserved warm/heat sensibility indicated an intact C-fiber system [35–37]. Similar allodynia was evoked by another innocuous stimulus, that is, vibration, and, akin to brushing, this effect also persisted when the myelinated fibers were blocked. That compression block of myelinated fibers fails to block this form of allodynia was tested and confirmed in multiple studies (overall n > 70 participants) [33,34,38–41], nonetheless, it is possible that some residual fibers in the myelinated range may still be intact despite the loss of perceptiveness to vibration and innocuous cold. Conversely, when the C fibers were preferentially blocked using a small amount of low-dose anesthetic injected intradermally at the site of the vibration probe (efficacy confirmed by the abolition of warm sensation and preserved cold and vibration sensations [36,37]), the vibration-evoked allodynia was abolished regardless of whether the myelinated fibers were conducting or not [33,34,38]. From here on, this

Dorsal horn circuitry targeted by C-LTMRs.

C-LTMR connections are indicated in blue. Green and red structures indicate excitatory and inhibitory neurons and connections, respectively. CR, calcitonin gene-related peptide neuron; GRP, gastrin-releasing peptide neuron; P, projection neuron; PKC\(_{\gamma}\), protein kinase \(\gamma\) neuron; PV\(_{\delta}\), excitatory parvalbumin neurons; PV\(_{\alpha}\), inhibitory parvalbumin neurons; ROR\(_{\beta}\), neuron expressing retinoid-related orphan receptor \(\beta\); TC, transient central cell; V, vertical cell. C-LTMRs provide input to some of the same neurons as low-threshold A fibers, forming a circuit that has been proposed to be involved in mechanical allodynia by relaying tactile input to lamina I projection neurons when PV\(_{\delta}\) neurons are inhibited [24]. Note that second-degree connections are tentative; for example, while C-LTMRs provide sparse synaptic input to CR neurons and CR neurons connect to lamina I projection neurons, the CR population is heterogeneous, and it is not known whether CR neurons specifically contacted by C-LTMRs establish connections with projection neurons. Also note that the neurons pictured may denote partly overlapping populations; for instance, some GRP neurons may be TC cells [58].

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**Figure 1**

To supraspinal sites

C-LTMR fibers

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phenomenon is referred to as C-LTMR-mediated alldynia for three reasons: the increase in pain was evoked by a non-nociceptive stimulus (brush/vibration), hence suggesting a low-threshold neural substrate; the allodynia persisted when the myelinated fibers were blocked but when the C fibers were blocked, the allodynia was abolished regardless of whether the myelinated fibers were conducting or not; the C-LTMR is the only known type of mechano-sensitive C fiber in the low-threshold domain. The onset of C-LTMR-mediated allodynia tended to be delayed by approximately 10–15 s after stimulus onset, the reason for which is not entirely clear but suggests a dependence on slow-conducting fibers and a possible need for temporal summation.

The C-LTMR-mediated alldynia appeared to be much less somatotopically constrained than Aβ-mediated alldynia [e.g. Ref. 42] as it could be reliably evoked across spinal segments/nerve types (ulnar/median/radial) and skin types (hairy/glabrous) [34], thus more closely mimicking pathological conditions in which pain does not always follow an orderly somatotopic pattern and regions beyond the site of injury can be affected [e.g. Ref. 43]. Much of the work on C-LTMRs has focused on hairy skin and whether they innervate glabrous skin is less clear, however, several recent studies point to the existence of a low-threshold C-fiber substrate in glabrous skin [34,44–46]. Indeed, the expression of alldynia was identical in human hairy and glabrous skin — effects that persisted during a myelinated-fiber blockade but were abolished by a C-fiber blockade [33,34].

The C-LTMR-mediated alldynia was not limited to the presence of tonic muscle pain and could be evoked during tonic cutaneous pain induced by a hypertonic-saline infusion [38]. In fact, while background nociceptive input was essential to generate central sensitization (a state of heightened excitability and synaptic efficacy of central nociceptive pathways [47]) and unmask the allodynic effect of C-LTMR activation, pain perception was not always necessary, as confirmed by observations in a model of delayed onset muscle soreness induced by uncustomed eccentric exercise in healthy participants [39]. In this model, there is no pain at rest (baseline pain rating = 0) and yet when vibration was applied to the skin overlying the sore muscle (soreness confirmed by a drop in pressure-pain thresholds), it was perceived as painful (alldynia) — an effect that persisted during compression block of myelinated fibers but was abolished by an anesthetic block of C fibers at the site of vibration. Further, it was possible to evoke alldynia in a patient with activity-triggered heel pain (no resting pain) without the need for experimental eccentric contractions. Akin to the healthy participants, the alldynia in the patient was evoked regardless of whether the myelinated fibers were conducting or not but was markedly reduced by anesthesia of C fibers [39]. These observations do not argue against the involvement of Aβ fibers in alldynia and it might be that the relative contribution of each fiber class is context-dependent and stimulus-dependent. Among the unmyelinated population, it is tempting to suggest that tactile and nociceptive fibers exhibit a kind of dualism: while the classical nociceptors are high-threshold mecanoreceptors that encode noxious stimuli [2,48] and convey a perceptible and qualitatively distinct aspect of pain [49], C-LTMRs may be more concerned with subperceptual processing but under certain conditions, they may contribute to a perceptual outcome, for example, alldynia during background nociceptive input [33,34,38,39].

In addition to C-LTMR-mediated alldynia evoked by touch, similar effects to cooling have been reported in healthy participants and a small number of patients with chronic pain [40]. The cold alldynia persisted during conduction block of myelinated fibers and desensitization of the heat-sensing capsaicin-sensitive transient receptor potential vaniloid 1 (TRPV1) and cold-sensing menthol-sensitive transient receptor potential subfamily 8 (TRPM8) channels but was abolished by blockade of low-threshold calcium channel CaV3.2 — a finding consistent with observations in mice that cold (and mechanical) alldynia is linked to the selective expression of CaV3.2 channels on C-LTMRs [50]. Further, in a study on touch perception, the capacity of healthy participants to detect innocuous mechanical indentation on the skin (applied using monofilaments) was largely preserved during a conduction block of myelinated fibers but the use of a CaV3.2 antagonist (while the myelinated-fiber block was still in place) resulted in a complete loss of innocuous monofilament sensibility adding further support to the selective expression of CaV3.2 on C-LTMRs [44].

Antinociceptive role
In contrast to a pronociceptive role, an antinociceptive role has also been suggested for C-LTMRs. In particular, TAF4A, a chemokine-like protein expressed by C-LTMRs, has been found to reduce mechanical allodynia in neuropathic and inflammatory pain models in rodents, possibly via a microglia-mediated reduction in excitability of dorsal horn neurons [51,52]. Interestingly, loss of bhlha9, a transcription factor highly enriched in C-LTMRs increases formalin-induced pain behavior in male but not female mice [53].

In human psychophysical studies, a comparison between brushing speeds reveals an important role of tactile affective valence in pain modulation. For instance, in a model of experimentally induced acute/short-lasting heat pain, when brushing was delivered at a stroking speed perceived as pleasant it reduced pain (an effect associated with low anxiety and high calmness levels), but when brushing was delivered at a speed perceived as less pleasant or neutral no analgesia was seen [54]. In a
hypertonic saline model of tonic muscle pain, whether tactile stimulation resulted in an excitatory or inhibitory effect on pain was linked to the affective tactile attribute: a single velvet-fabric stroke, perceived as pleasant, reduced pain, and both the pleasantness of velvet fabric and the analgesia persisted during a conduction block of myelinated fibers; a single sandpaper stroke, perceived as unpleasant but not painful (rated as naught on a pain scale, no visible signs of skin abrasion, and the unpleasantness rating returned to neutral before the next trial), exacerbated tonic muscle pain, and both the unpleasantness of sandpaper and the allodynia persisted during a conduction block of myelinated fibers [41]. These observations on bidirectional affect-based modulation of pain indicate a dual function within the C-fiber domain. A potential limitation of this study is that while the sandpaper chosen was fine (300 grit) and perceived as non-painful it still cannot be ruled out that the high-threshold receptors/nociceptors were not activated, however, similar affect-based modulation driven by C fibers was shown using vibration — a clearly non-nociceptive stimulus — by varying the frequency parameter [41] highlighting the complexity of the afferent coding of affect. How affect is encoded in the periphery is unclear; that stroking is effective suggests the need for progressive recruitment of multiple units over a large area; that vibration can also be effective suggests a coding strategy based on an impulse pattern at a fixed point of contact. Further research is clearly needed to understand the afferent coding of affect.

Conclusions and future directions

In summary, the evidence regarding pro-nociceptive or anti-nociceptive functions of C-LTMRs may appear somewhat contradictory. However, it is possible that these conflicting observations could be attributed to dual pro-nociceptive or anti-nociceptive functions of C-LTMRs. Indeed, certain features of the C-LTMR system uncovered in rodents strongly suggest the potential for C-LTMR-mediated bidirectional modulation of nociceptive signals and pain perception. For instance, C-LTMRs target PKγ neurons which are thought to play a key role in mediating mechanical allodynia in inflammatory and neuropathic conditions [23]. Moreover, while sparse, synaptic input from C-LTMRs to calretinin neurons could contribute to mechanical hypersensitivity, as such neurons have been implicated in inflammation-induced allodynia and nocifensive behavior [29,55–57]. Conversely, activation of GABAergic parvalbumin neurons may alleviate allodynia [24]; C-LTMR input to such neurons may provide a potential synaptic substrate for touch-mediated attenuation of pain, in addition to the more broadly acting volume transmission afforded by TAF/A4. Moreover, the complex synaptic arrangements of C-LTMRs in rodents suggest a capacity for a wide range of synaptic plasticity mechanisms of C-LTMR-mediated input to the dorsal horn circuitry; presynaptic modulation of C-LTMR terminals can both inhibit and enhance C-LTMR synaptic transmission, whereas selective plasticity of C-LTMR synapses onto different classes of interneurons may provide differential modulation of subcircuits of the nociceptive and wider somatosensory circuitry of the dorsal horn. Clearly, future studies are needed to further clarify these issues. Furthermore, given the paucity of translational success in the pain field, an important aim for future research should be to relate existing observations in rodents to primates and humans and vice versa. Increased understanding of the role of this enigmatic fiber system in pain regulation in humans and animals could provide a basis to identify new therapeutic targets for more effective and selective modes of pain control.

Author contributions

ML and SSN contributed equally to the preparation of the article and have approved the submitted version.

Conflict of interest statement

Nothing declared.

Acknowledgement

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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