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Research paper

Electrophysiological evidence for the existence of a rare population of C-fiber low threshold mechanoreceptive (C-LTM) neurons in glabrous skin of the rat hindpaw

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HIGHLIGHTS

- C-LTMs (C-mechanoreceptors) are thought to exist only in the hairy skin.
- Direct evidence that C-LTMs exist in glabrous skin of rat hindpaw is provided.
- They constituted >6% of the C-fiber neurons with receptive fields in glabrous skin.
- Their mechanical and electrical thresholds are much lower than those of C-nociceptors.
- They have faster action potential kinetics than C-nociceptors.

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ABSTRACT

The mammalian skin in innervated by distinct classes of low-threshold mechanoreceptive (LTM) primary afferent neurons that are classified as Aβ-, Aδ- or C-LTMs according to their axonal conduction velocities (CVs). C-LTMs are thought to signal pleasant and erotic touch sensations in humans, and to exist only in the hairy skin of primates and other species. Using intracellular recordings from rat L4/L5 dorsal root ganglion (DRG) neurons that were classified in vivo as C-nociceptors or C-LTMs, according to their dorsal root CVs and their responses to mechanical and thermal stimuli, the present study provides the first electrophysiological evidence that C-LTMs exist in the glabrous skin of the rat's hindpaw. Indeed 6.4% (5/78) of the total sample of lumbar C-fiber DRG neurons with receptive fields in the glabrous skin of the rat hindpaw were C-LTMs. The electrophysiological properties of this rare subpopulation of C-fiber neurons (mean CV = 0.48 ± 0.06 m/s) are distinct from those of C-fiber high threshold mechanoreceptors (HTMs). Indeed, their mean mechanical (1.7 ± 1.1 mN) and electrical (4.0 ± 0.4 V) thresholds was significantly different from that of C-HTMs. They also exhibited faster action potential and afterhyperpolarization kinetics than C-HTMs. The present study lends support to previous studies that have provided indirect evidence for the presence of C-LTMs in glabrous skin. If C-LTMs are present in human glabrous skin, they may, in this type of skin, represent a novel peripheral neuronal substrate for the pleasant/social touch sensation, and account for or contribute to touch hypersensitivity after injury.

1. Introduction

The mammalian skin is innervated by a variety of morphologically and physiologically distinct classes of primary afferent neurons that conduct sensory information from the periphery to the CNS (e.g., see Ref. [1]). Activation of these sensory neurons, whose somata reside within (spinal) dorsal root ganglia (DRG) or trigeminal (cranial) ganglia, is the first step in any somatosensory perception. The perception of gentle touch of the skin is mediated by distinct classes of low-threshold mechanoreceptive (LTM) primary afferent neurons that are classified, according to their axonal conduction velocities (CVs), as Aβ-, Aδ- or C-LTMs (see e.g., Refs. [1,2]). C-LTMs (also known as C-mechanoreceptors) were first described in the cat [3], and were subsequently found in the hairy skin of various species including mouse (e.g., Ref. [4]), guinea pig (e.g., Refs. [5,6]), rat (e.g., Refs. [7,8]); cat (e.g., Ref. [9]), pig [10] and monkey (e.g., Ref. [11]). However for several decades, C-LTMs were thought to be lacking entirely in human skin, but in late 1980's a microneurographic study by Johansson et al. [12] showed that they

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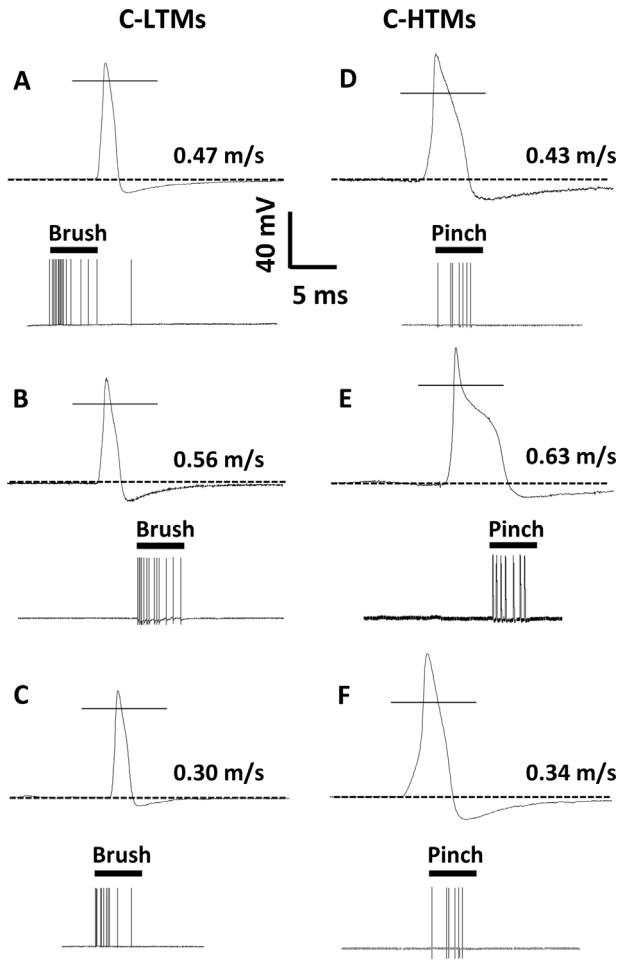

Fig. 1. Examples of somatic APs recorded from C-LTMs and C-HTMs with receptive fields in glabrous skin of rat hindpaw. Shown on the left panel (A–C) are examples of APs recorded from three C-LTMs that responded to stroking the skin slowly and briefly with a paint brush. The responses to the brush (about 1 s) are shown in the bottom traces. Shown on the right panel (D–F) are examples of APs recorded from C-HTMs that responsed to a brief noxious pinch (but not to innocuous stimuli including brush, touch, pressure, and tap). The responses to the brief noxious pinch (about 1 s) is shown on the bottom traces. The CV of each neuron is given to the right of the AP. The solid lines crossing the APs show 0 mV. Note that the AP duration and AP overshoot, and AHP duration in C-LTMs are smaller/shorter than those in C-HTMs. Note that the time and voltage scales apply only to the top traces.

do exist in human hairy skin. More recent microneurographic studies have shown that C-LTMs are encountered, in the human hairy skin, almost as frequently as the A-fibers [13,14] (see also Ref. [15] for review).

C-LTMs are exquisitely sensitive to indentation of the hairy skin, but respond optimally to light (innocuous) and slow stroking of the skin at a velocity of 1–10 cm/s, and to innocuous cooling (e.g., Refs. [4,16,17]). Although their functional role is still largely unknown, C-LTMs (known as C-tactile afferents in humans) have been implicated in itch and tickle sensations, pleasant/social and erotic touch, and injury-induced mechanical hypersensitivity [4,13,18] (see Ref. [15] for review).

There is no direct evidence yet for the existence of C-LTMs in the glabrous skin of primates or other species, but several lines of indirect evidence suggest that the presence of a functionally equivalent class to the C-LTMs in the glabrous skin is highly plausible (see Section 4). Using intracellular recordings from rat L4/L5 DRG neurons that were classified in vivo as C-nociceptors or C-LTMs, according to their dorsal root CVs, and their responses to mechanical and thermal stimuli, the present electrophysiological study provides the first direct evidence for the existence of a small population of C-LTMs in the glabrous skin of the rat's hindpaw.

2. Materials and methods

2.1. Experimental animals

Young female Wistar rats (180–220 g, Charles River, U.K.) were used; they were housed in a room maintained at room temperature between 22 and 24 °C while under a 12-hour (h) dark and light cycle, with soft bedding and access to food and water *at libitum*. All experimental procedures were approved by the University of Liverpool Ethical review group, and complied with the 1986 UK Scientific Procedures Animals Act.

2.2. In vivo electrophysiological experiments

The experiments were conducted as described previously [6,8,19] under deep anesthesia induced with an initial dose of sodium pentobarbitone (60 mg/kg, i.p.). Core temperature was maintained at ~36 ± 0.5 °C, and the temperature in the paraffin pool measured near the DRG under study was maintained throughout close to 30 ± 2.0 °C. Intracellular voltage recordings were made from neuronal somata of L4/L5 C-fiber DRG neurons using sharp glass microelectrodes filled with 1 M KCl, (see Refs. [19,20]).

2.2.1. Conduction velocity (CV) classification

Somatic action potentials (APs) evoked antidromically by stimuli applied to the dorsal root were recorded on line, but analyzed offline using the CED Spike II program and scripts (see Refs. [21–23]). The CV for each neuron was estimated (offline) by dividing the conduction distance by latency (in ms) from the stimulus artifact to the AP onset. C-fiber neurons were those with a dorsal root CV of ≤0.8 m/s (see Ref. [24]). Various electrophysiological variables were measured (see Ref. [6]).

2.2.2. Functional classification of the DRG neurons

Hand-held natural stimulators were applied to the glabrous skin of the ipsilateral (left) hindpaw, to evaluate the sensory modality of each recorded neuron. As described previously [19–24] C-nociceptive neurons were those that failed to respond to low-intensity (non-painful) stimuli (e.g., light pressure or stroking the skin with a fine paint brush), but responded to noxious mechanical stimuli applied using fine- or coarse-toothed forceps or sharp objects (e.g., needles) and/or noxious thermal stimuli including heat (hot water at >50 °C) and ice (applied for 5–10 s). The nociceptive neurons included: (1) high-threshold mechanoreceptive (HTM) units that responded only to noxious mechanical stimuli or had deep (subcutaneous) mechanical receptive fields (RFs) and were, therefore, not tested with thermal stimuli, (2) mechano-heat (MH) and mechano-cold (MC) units with superficial or dermal RFs that responded respectively to both noxious mechanical stimuli and to a single application of noxious heat (MC units) or noxious cold (MC units). In contrast, C-LTMs were those neurons that responded to the low intensity (innocuous) stimuli; they responded preferentially to gentle contact moving very slowly across the skin at speed of a few mm/s, and sometimes to cooling as previously reported in several species (e.g., Refs. [1,2,25]).

The mechanical thresholds were determined for all C-LTMs ($n = 5$) and some C-HTMs ($n = 23$) using calibrated von Frey filaments. These were applied perpendicularly to the most sensitive spots in the receptive fields of these neurons for 5–10 s. The response threshold was defined as the lowest von Frey filaments force (in mN) needed to evoke at least 2 APs during the application period (see Refs. [7,20]). The dorsal root electrical threshold