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Research article

Mice and rats differ with respect to activity-dependent slowing of conduction velocity in the saphenous peripheral nerve

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HIGHLIGHTS

- We assessed in mice the a published criteria for C fibre differentiation into sub-classes.
- For this, we developed an *in vivo* electrophysiological technique in the mouse.
- The criteria for differentiation into fibres sub-classes is invalid in the mouse.

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ABSTRACT

We assess in mice, the electrophysiological criteria developed in humans and rats *in vivo* for unmyelinated (C) fibre differentiation into sub-classes, derived from the activity-induced latency increase ("slowing") in response to electrical stimulation during 6 min at 0.25 Hz followed by 3 min at 2 Hz. Fibres are considered nociceptors if they show more than 10% slowing at 2 Hz; nociceptors are further divided into mechanosensitive ("polymodal") and mechanoinsensitive ("silent") ones according to a latency shift of less and more than 1% during the first minute at 0.25 Hz, respectively. Sympathetic postganglionics are recognised by 2–10% slowing at 2 Hz; units slowing less than 2% at 2 Hz remain uncategorised.

For assessment of these criteria, we also developed a novel *in vivo* technique for recording of peripheral single-fibres in the mouse.

We compared the theoretical slowing-rate discriminator criteria with experimental data obtained from mice *in vivo/in vitro* and rats *in vitro*. Out of 69 cutaneous mouse C-fibres *in vitro* and 19 *in vivo*, only 38 (67%) and 9 (47%) met the above 1% criterion, respectively; sympathetics were not identified. In contrast, out of 20 rats nerve fibres *in vitro*, 19 (95%) met this criterion.

We conclude that (A) our novel electrophysiological technique is a practical method for examining mouse cutaneous single-fibres *in vivo* and (B) the published criterion for identifying silent nociceptors in rats and humans is not applicable in mice.

1. Introduction

Neuronal electrophysiological recordings on single-fibre level are a useful tool in the research on various neuropathies. They allow refined examination of the changes in many receptive and conductive properties under pathological conditions and moreover, enable linking specific fibre types to these conditions. This has proven significant, with various pathologies being connected to particular subsets of fibres. For instance, mechanical hypersensitivity induced by partial nerve injury has been shown to be signaled by myelinated (A) fibres, whereas specific unmyelinated (C) fibres are responsible for concomitant heat sensitisation and pain [1,2]. The discrepancies between mechanically sensitive and insensitive (C) fibres seem to be of particular importance. Mechanically, insensitive ("silent") fibres have previously been connected to chronic pruritus and erythromelalgia in human patients [3,4].

The classification into specific neuronal fibre types by electrophysiological recordings can be made using a combination of conductive and receptive criteria. Receptive characterization could be achieved through screening for heat, cold and mechanosensitivity and a conductive index includes velocity of action potential (AP) conduction and changes in conduction velocity in response to

Abbreviations: AP, action potential; C-HTMs, C high threshold mechanosensitive fibre; C-LTMs, low threshold mechanosensitive C-fibre; CMi, mechanoinsensitive C-fibre; CH, heat-sensitive C-fibre; CMC, mechanosensitive cold C-fibre; CMH, mechanosensitive heat C-fibre; CMHC, mechanosensitive heat and cold C-fibre; CMs, mechanosensitive C-fibre; RF, receptive field.

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repetitive electrical stimulation. Such velocity changes are utilised in the "collision technique", first described by Iggo in the cat [5], in which activity-induced slowing of conduction velocity during repetitive electrical stimulation is used to identify discrete fibre units. Since then, distinct activity-induced changes in conduction velocity have been established as a marker for specific fibre subsets in rats and humans [6–9].

A particularly elaborated index for fibre screening through activity-induced conduction velocity changes (termed "slowing" and "speeding") has been established *in vivo* by Hugh Bostock and collaborators. A specific electrical stimulation protocol was shown to be discriminative between specific peripheral nerve fibre subclasses in both rats [10] and humans [11]. The protocol entails quiescence for 3 min, followed by 6 min of electrical stimulus at 0.25 Hz, 3 min at 2 Hz and again 6 min at 0.25 Hz. The first fibre differentiation into nociceptive, sympathetic postganglionic and uncategorised nerve fibres is based on activity-induced latency increase by more than 10%, 2–10%, or less than 2%, respectively, during the 3 min electrical stimulation at 2 Hz. A further sub-division of nociceptors is based on the conduction slowing during the first minute at 0.25 Hz following the initial rest period. Fibres slowing more than 1% (rat criterion, human criterion is 1.5%) during this period are classified as mechanoinsensitive, as opposed to mechanosensitive ones which slow less than 1%.

We have tried to apply these published criteria to the mouse. For this, we have also developed a novel technique for *in vivo* electrophysiological recording on single-fibre level in the mouse, which is an adaptation of an *in vitro* recording method used for neuronal recordings from rat cranial meninges [12].

2. Methods

2.1. Animals

C57BL/6 mice and Wistar rats of both sexes and ranging in weight between 20–27 g and 200–300 g, respectively, were housed in group cages in a temperature-controlled environment with a 12 h light–dark cycle and were supplied with food and water *ad libitum*. All animal experiments were carried out in compliance with the local Animal Protection Authority.

2.2. In vitro electrophysiology

Animals were sacrificed by exposure to a rising CO_2 atmosphere. Single-fibre recordings from cutaneous (C) fibres of the saphenous nerve were obtained using an isolated skin–nerve preparation essentially as described previously [13–15], using a CED Micro1401 and Spike2 software for digital storage and evaluation (Cambridge Electronics, UK). Mechanosensitive receptive fields were searched for using a blunt glass rod, and the mechanical threshold was assessed using calibrated von Frey bristles. Cold stimuli (flushing with ice-cold buffer) as well as radiant heat stimulation (20 s ramp of 32–48 °C delivered by a feedback-controlled halogen lamp with an 8 mm focused beam) were applied. Heat threshold was defined as the temperature at which the second spike occurred. Following sensory characterisation, the receptive field (RF) was electrically stimulated using a metal electrode as cathode, and fibres were characterised using a specific electrical protocol consisting of 3 min quiescence, 6 min at 0.25 Hz followed by 3 min at 2 Hz and again 6 min at 0.25 Hz. The activity-induced latency shifts during 2 Hz and the first minute at 0.25 Hz were used for differentiation of the fibres into sub-classes as previously published [11,16]. Mechanoinsensitive C fibres were searched for using a hand-held metal electrode delivering supramaximal electrical pulses along the nerve arborization and into the skin, as described [14]. Once the termination of a unit in the skin was recognised, conduction velocity was determined and a marking technique, using simultaneous electrical stimulation of the terminal and the main nerve trunk was applied, to ensure recording from a single unit. The cold, heat and electrical stimulation procedures that followed were identical to the ones used for mechanosensitive fibres.

2.3. In vivo electrophysiology

Mice were initially anaesthetised in an isoflurane–oxygen atmosphere (2%) to produce deep aneasthesia with absence of limb withdrawal reflexes upon pinching with forceps. Mice were then placed on their backs, continuously supplied with an isoflurane–oxygen mixture through a custom-made perspex mask and their hind legs were fixed to a perspex block using double-sided adhesive tape. Body temperature was maintained at 37 °C with a feedback-controlled homoeothermic system (TKM 0902; Föhr Medical Instruments, Frankfurt, Germany) containing a rectal thermosensor (pt100) of 1 mm diameter. A small incision of approximately 2.5 mm diameter was made on the medial side of the hind limb at knee level, the appearing saphenous nerve was freed from its surrounding tissue and cut. The isoflurane concentration

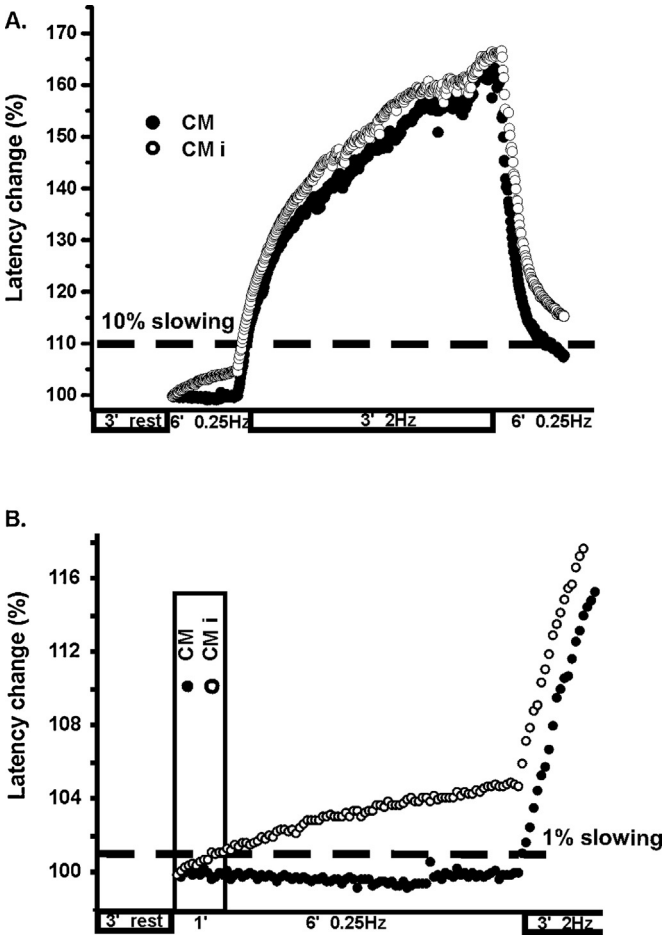

Fig. 1. (A) Depicts the electrical protocol used for classification of fibre subsets and the activity-induced slowing profile (dot plot) of one mechanosensitive (CM, filled spheres) and one mechanoinsensitive (CMi, empty spheres) examples. Fibres with a latency shift of more than 10% (hatched line) during the 2 Hz period should be nociceptors according to slowing criterion. (B) Illustration of the differentiating criterion between mechanically sensitive and insensitive fibres. The activity-induced latency shifts of the two units shown in 1(A) during the initial part of the electrical protocol are depicted. The 1% slowing criterion during the first minute (highlighted) of stimulation at 0.25 Hz is shown as a hatched line.