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Low-Frequency Stimulation of Silent Nociceptors Induces Secondary Mechanical Hyperalgesia in Human Skin

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Abstract—Secondary mechanical hyperalgesia to punctate mechanical stimuli and light touch (allodynia) are prominent symptoms in neuropathic pain states. In a combined microneurographic and psychophysical study, we investigated the role of mechano-insensitive (silent) nociceptors regarding induction. Electrical thresholds of mechano-sensitive and silent nociceptors were assessed by microneurography with two closely spaced *intracutaneous* electrodes (i.c.) and a *transcutaneous* configuration (t.c.) in the foot dorsum. For t.c. stimulation there was a marked difference between silent (median, quartiles; 60, 50–70 mA, $n = 63$) and mechano-sensitive fibers (3, 2–5 mA, $n = 107$). In silent fibers, thresholds were lower for i.c. stimulation (16, 14–19 mA, $n = 8$), but higher in mechano-sensitive units (6, 5–6 mA, $n = 13$). Corresponding psychophysical tests showed no difference between the stimulation configuration for pain thresholds, but lower thresholds for the i.c. stimulation in axon reflex erythema (12 vs. 21 mA), punctate hyperalgesia (9 vs. 15 mA) and allodynia (15 vs. 18 mA). Punctate hyperalgesia was evoked at very low stimulation frequencies of 1/20 Hz (7/7 subjects), whereas the induction of an axon reflex flare required stimulation at 1/5 Hz. Electrical stimulation which is sufficient to excite mechano-insensitive C nociceptors can induce secondary mechanical hyperalgesia even at low frequencies supporting a role of such low-level input to clinical pain states. Thus, differential nociceptor class-specific input to the spinal cord adds to the complexity of modulatory mechanisms that determine nociceptive processing in the spinal cord.

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INTRODUCTION

Sensitivity to noxious and non-noxious stimulation is often increased in chronic neuropathic pain conditions, based on sensitization of spinal processing (Finnerup et al., 2016) and resulting in mechanical hyperalgesia to pinprick (“punctate hyperalgesia”) and to brush (“allodynia”) is observed in about 20% of neuropathic pain patients (Baron et al., 2017). Experimentally, secondary mechanical hyperalgesia can be induced by algogens such as capsaicin (Simone et al., 1989; LaMotte et al., 1991), mustard oil (Koltzenburg et al., 1994) or noxious heat (Moiniche et al., 1993) or by electrical stimulation (Dusch et al., 2010; Henrich et al., 2015) with stimulation frequencies varying between 1 and 100 Hz. Typically, high-frequency bursts (100 Hz) of primary afferent C fibers are used to induce activity-dependent increases of synaptic strength (long-term potentiation – LTP) of nociceptive spinal synapses (Sandkuhler, 2007). In contrast, capsaicin application provokes long-term discharge at moderate frequencies in mechano-insensitive nociceptors rather than in polymodal nociceptors (Schmelz et al., 2000b; Wooten et al., 2014). The mechano-insensitive (“silent”) C-nociceptors are also involved in the induction of axon reflex flare (Schmelz et al., 2000a), and notably spontaneous discharge was found to be higher in patients with neuropathic pain only in this nociceptor population (Kleggetveit et al., 2012; Serra et al., 2012; Serra et al., 2014). However, spontaneous activity in C-nociceptors under neuropathic condition was found to be very low (few impulses per minute) also in experimental pain models (Wu et al., 2001, 2002) and therefore seems unlikely to contribute significantly to nociceptive processing. On the other hand, even low-frequency stimulation (1 Hz) at

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C-fiber strength can be sufficient to cause LTP (Liu and Sandkuhler, 1997; Ikeda et al., 2006; Drdla and Sandkuhler, 2008) of spinal cord neurons in rodents. Thus, even low-level activity of C-nociceptors can modulate spinal processing, in particular when differential nociceptor classes are taken into account.

We therefore assessed electrical thresholds of silent and polymodal nociceptors for *intracutaneous* and *transcutaneous* stimulation by microneurography and used both stimulation paradigms to assess the intensity-dependent induction of mechanical hyperalgesia and neurogenic inflammation in healthy volunteers. Using the optimum stimulation paradigm, we also assessed the minimal frequency required to induce axon reflex flare, punctate hyperalgesia and touch-evoked allodynia in human skin. Punctate hyperalgesia was evoked by stimulating silent nociceptors at an unexpectedly low frequency of 1/20 Hz, thereby confirming a possible pathophysiological role of low level discharge in C-nociceptors for the induction of sensitized spinal processing.

EXPERIMENTAL PROCEDURES

Subjects

In the experimental protocols 81 healthy volunteers aged 22–34 years (39 females 46 males) participated after having given their informed consent. All subjects were familiar with the principles of the method and the general intention of the study, but were naive to the specific experimental goals. The study was approved by the local ethics committee in Erlangen. The volunteers were comfortably seated on a reclining chair and their left arm or left foot was placed in a vacuum cushion with the volar side up. The limb was stabilized to keep it in the same relaxed position during the whole experiment. Experimental sessions lasted about two and a half hours.

Electrical stimulation

For *intracutaneous* stimulation, stainless steel wires (diameter 0.1 mm) were inserted at a depth of 1 mm over a length of 1 cm by use of a 25G canula. To avoid direct contact with the tissue, the wires were inserted in plasmapheresis fibers (Asahi, outer diameter 0.4 mm) which were perfused with isotonic saline at a flow rate of 4 μl/min. Two capillaries were inserted in parallel at a distance of 2 mm (Fig. 1) in the dorsum of the foot or the central volar forearm. For *transcutaneous* stimulation, a circular surface electrode (5 mm diameter) served as cathode with a large stainless steel surface electrode (4 * 7 cm) attached at a distance of 30 cm as anode. To reduce skin resistance, electrode paste was used for *transcutaneous* stimulation.

Sixty minutes after the insertion, electrical stimulation was applied for 30 or 90 min via a constant current stimulator (Digitimer Ltd., DS7, Welwyn-Garden, UK). Current intensity was gradually increased to a minimum of 25 mA during the first 10 min of the stimulation aiming at a pain rating of six out of ten (see below). Maximum stimulus intensity was 60 mA or a pain rating

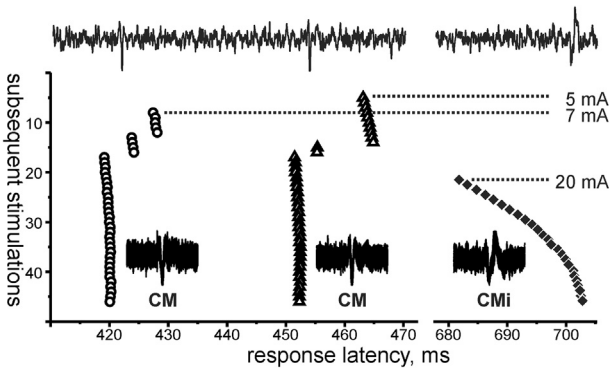

Fig. 1. Electrical thresholds of C-nociceptors Microneurographic recordings (original recording, top panel) of individual C fibers responding to electrical stimulation inside their innervation territory in the dorsum of the foot. Stimuli were applied at a frequency of ¼ Hz and responses are shown in subsequent traces from top to bottom. Mechano-responsive units (CM) are depicted with open symbols, the mechano-insensitive fiber (CMI) with filled diamonds. *Intracutaneous* stimulation was applied inside the innervation territory at increasing intensity. The lowest stimulation intensity required to activate the unit was assessed (*intracutaneous* electrical threshold; 5, 7, 20 mA). Note that with increasing stimulation intensity response latency of the mechano-responsive units "hobs" (Peng et al., 1999) to an earlier one when a faster conducting branch is recruited. Shapes of action potentials of the units are depicted superimposed in the insets.

of eight out of ten. To assess intensity-response relation, the current was increased from 0 in 3 mA steps (0.5 ms pulse duration) every 10 min to a maximum of 30 mA in the trans- and *intracutaneous* setting. In another protocol, assessing effects of different stimulation frequencies, immediately following insertion of the membranes, the current intensity evoking a pain rating of seven out of 10 was assessed. Low-frequency stimulation commenced at this intensity after a baseline of 60 min. Stimulation frequency was increased stepwise from 1/min to 2/min, 3/min, 6/min and 12/min, with each frequency being applied for 10 min.

In two separate protocols, we compared either regular 1/4 Hz stimulation (0.5 ms, 25 mA) with the stimulation of twin pulses (50 ms interval) every 8 s or regular 1-Hz stimulation with the stimulation of twin pulses (50 ms interval) every two seconds in a cross-over fashion at the right and left forearm of the volunteers for the induction of pain, axon reflex flare erythema and area of punctate hyperalgesia.

Psychophysics

The subjects were asked to rate the pain sensation induced by electrical stimuli on a numeric scale from 0 to 10, in which the value of 0 should indicate no pain and 10 should be assigned to the maximum pain the subject could imagine. In addition, areas of secondary hyperalgesia were determined at regular intervals. The area of touch-evoked mechanical hyperalgesia (allodynia) was assessed by lightly stroking the skin with a cotton swab. Strokes were applied at a rate of 1 Hz and started well outside the area of allodynia approaching the stimulation site from at least eight different directions. The distance at which this

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