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RESEARCH ARTICLE

Nociception induces a differential presynaptic modulation of the synaptic efficacy of nociceptive and proprioceptive joint afferents

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Abstract

A previous study has indicated that during the state of central sensitization induced by the intradermic injection of capsaicin, there is a gradual facilitation of the dorsal horn neuronal responses produced by stimulation of the high-threshold articular afferents that is counteracted by a concurrent increase of descending inhibitory actions. Since these changes occurred without significantly affecting the responses produced by stimulation of the low-threshold articular afferents, it was suggested that the capsaicin-induced descending inhibition included a preferential presynaptic modulation of the synaptic efficacy of the slow conducting nociceptive joint afferents (Ramírez-Morales et al., Exp Brain Res 237:1629–1641, 2019). The present study was aimed to investigate more directly the contribution of presynaptic mechanisms in this descending control. We found that in the barbiturate anesthetized cat, stimulation of the high-threshold myelinated afferents in the posterior articular nerve (PAN) produces primary afferent hyperpolarization (PAH) in the slow conducting (25–35 m/s) and primary afferent depolarization (PAD) in the fast conducting (40–50 m/s) articular fibers. During the state of central sensitization induced by capsaicin, there is a supraspinally mediated shift of the autogenic PAH to PAD that takes place in the slow conducting fibers, basically without affecting the autogenic PAD generated in the fast conducting afferents. It is suggested that the change of presynaptic facilitation to presynaptic inhibition induced by capsaicin on the slow articular afferents is part of an homeostatic process aimed to keep the nociceptive-induced neuronal activity within manageable limits while preserving the proprioceptive information required for proper control of movement.

Keywords Articular afferents · Capsaicin · Nociception · Descending presynaptic inhibition · Primary afferent depolarization

Introduction

We have shown time ago that in the anesthetized cat, the intradermal injection of capsaicin facilitated the dorsal horn neuronal responses produced by stimulation of the high-threshold myelinated afferents in the posterior articular nerve (PAN). Quite interestingly, this facilitation lasted only a couple of hours, in spite of the persisting paw inflammation. The decline of the capsaicin-induced facilitation was attributed to increased descending inhibitory influences that prevented excessive activation of dorsal horn neurons during nociception (Rudomin and Hernández 2008).

More recently, we examined the effects of successive reversible spinal cold blocks on the PAN responses evoked in the dorsal horn before and after the intradermal injection of capsaicin (Ramírez-Morales et al. 2019). We found that after this nociceptive stimulus, the PAN responses produced by activation of high-threshold myelinated fibers increased gradually during successive reversible spinal blocks, in contrast with the responses evoked by stimulation of intermediate and low-threshold afferents that were barely affected. To explain this dual action, we assumed that the nociceptive-induced increase in descending inhibition had a presynaptic component that reduced the synaptic efficacy of

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the high-threshold PAN afferents, basically without affecting the effectiveness of the low-threshold PAN afferents.

The observations we now present were aimed to test this proposal more directly by comparing the effects of the intradermal injection of capsaicin and of spinal cold block on the intraspinal threshold changes elicited in single PAN afferents by stimulation of other fibers in the same nerve (autogenic stimulation), as well as by stimulation of cutaneous afferents (heterogenic stimulation). We found that the intradermic injection of capsaicin induced a supraspinally mediated shift of the autogenic primary afferent hyperpolarization (PAH) displayed by the slowest (25–35 m/s) PAN afferents to primary afferent depolarization (PAD), in contrast with the autogenic PAD exhibited by the fastest (40–50 m/s) afferents that remained basically the same. In other words, capsaicin changed the presynaptic facilitation produced by joint nociceptive afferents on themselves to presynaptic inhibition without changing the information transmitted by proprioceptive afferents.

Altogether, the present observations indicate that the descending control activated by nociceptive stimulation includes feed-forward presynaptic mechanisms that reduce the effectiveness of joint nociceptive afferents as part of a homeostatic process aimed to keep neuronal activity within manageable limits while preserving the information transmitted by proprioceptive afferents, essential for proper control of movement.

We found in addition that during the state of central sensitization induced by capsaicin, there was a mild reduction of the PAD produced by cutaneous inputs on both the slow and fast joint afferents that was barely affected after capsaicin and during spinalization. These effects are envisaged as part of a process that allows skin afferents to modulate the information carried by the articular fibers, a feature of relevance for the execution of limb movements under normal conditions and also during inflammation.

Preliminary observations have been presented in abstract form (Ramírez-Morales et al. 2011, 2014).

Methods

Ethical approval

Cats were bred and housed under veterinarian supervision at the Institutional Animal Care unit (SAGARPA permission AUT-B-C-0114-007). They were kept in individual comfortable cages and had access to food and water ad libitum. All experiments were approved by the Institutional Ethics Committee for Animal Research (Protocol no. 126–03) and comply with the ethical policies and regulations (see Grundy 2015). The Guide for Care and Use of Laboratory Animals (National Research Council 2010) was followed in all cases.

General procedures

The experiments were carried out in 17 adult cats of either sex (2.4–4.6 kg). The animals were initially anesthetized with pentobarbitone sodium (40 mg kg^{-1} I.P.). The carotid artery, radial vein, trachea and urinary bladder were cannulated. Additional doses of pentobarbitone sodium were given intravenously to maintain a deep level of anesthesia, tested by assessing that withdrawal reflexes were absent, that the pupils were constricted and that arterial blood pressure was between 100 and 120 mm Hg. When necessary, dextran 10% or ethylephrine (Effortil, Boering-Ingelheim) was administered to keep blood pressure above 100 mm Hg. A solution of 100 mM of sodium bicarbonate with 5% glucose was given I.V. (0.03 ml min^{-1}) to prevent acidosis (Rudomin and Lomelí 2007).

The sural (SU), saphenous (Saph), peroneous superficialis (SP) and posterior articular (PAN) nerves in left leg were dissected free. The SU and PAN nerves were sectioned while the SP and Saph nerves were left intact. The lumbosacral and low thoracic spinal segments were exposed by a laminectomy.

After the surgical procedures, the animals were transferred to a stereotaxic metal frame allowing immobilization of the spinal cord, paralyzed with pancuronium bromide (0.1 mg kg^{-1}) and artificially ventilated. Tidal volume was adjusted to have a concentration of 4% CO_2 in expired air. To prevent desiccation of the exposed tissues, pools were made with the skin flaps, filled with paraffin oil and maintained between 36 and 37 °C by means of radiant heat.

Cord dorsum and intraspinal recordings

Cord dorsum potentials (CDPs) evoked by electrical stimulation of the dissected nerves were recorded by means of a series of silver ball electrodes placed on the L4-S1 segments. Recordings of the intraspinal field potentials (IFPs) and intraspinal microstimulation were done using glass micropipettes filled with a 2 M NaCl solution (1–2.2 MΩ, tip diameter 3–5 µm). The CDPs and field potentials were measured against a reference electrode placed in nearby paravertebral muscles (band pass filter between 0.3 and 10 kHz). The micropipettes were inserted in the segments where the CDPs evoked by PAN stimulation were maximal (see Rudomin et al. 2007; Ramírez-Morales et al. 2019).

Peripheral stimulation

The intact SP, Saph as well as the central end of the SU nerve were mounted on bipolar hook electrodes for stimulation. The central end of the PAN was mounted on two

pairs of bipolar hook electrodes, one for stimulation and the other one for recording antidromic action potentials generated by intraspinal microstimulation (see Fig. 1). The SP, Saph and SU nerves were stimulated using 0.1 ms single pulses applied 35 ms before the intraspinal excitability test stimulus. As in previous studies, for the conditioning stimulation of PAN (autogenic) we used trains of four pulses at 700 Hz, also applied 35 ms before the intraspinal stimulus (Rudomin and Hernández 2008; Ramírez-Morales et al. 2019). Stimulus intensities are expressed as multiples of the strengths required to activate the most excitable afferents in each nerve (xT).

During the experiment the cord dorsum potentials and the intraspinal field potentials were averaged on-line (16 samples at 1 Hz) and the results digitally stored in the computer memory for processing after the experiment.

Intraspinal threshold changes of single afferents

In each experiment, one or two recording micropipettes were inserted into the dorsal horn in segment L6 using as a guide the field potentials produced by stimulation of the PAN (see Jankowska et al. 1993; Rudomin et al. 2004; Rudomin and Lomelí 2007). Once in position, the recording micropipettes were connected to separate computer-controlled stimulators that generated constant current pulses (0.4 ms, 1–30 µA) at 1 Hz and were displaced until each of them produced all-or none antidromic responses in the PAN (see Fig. 1).

The occurrence of PAD and PAH was examined in each fiber by measuring the intraspinal threshold changes produced during conditioning stimulation of sensory nerves. When using two micropipettes, independent current pulses were applied through each of them and their strength was automatically adjusted by the computer to produce, in each case, antidromic responses of the afferent fiber with a probability of 0.5 (see Madrid et al. 1979). The current pulses were delivered once per second, integrated, and the obtained

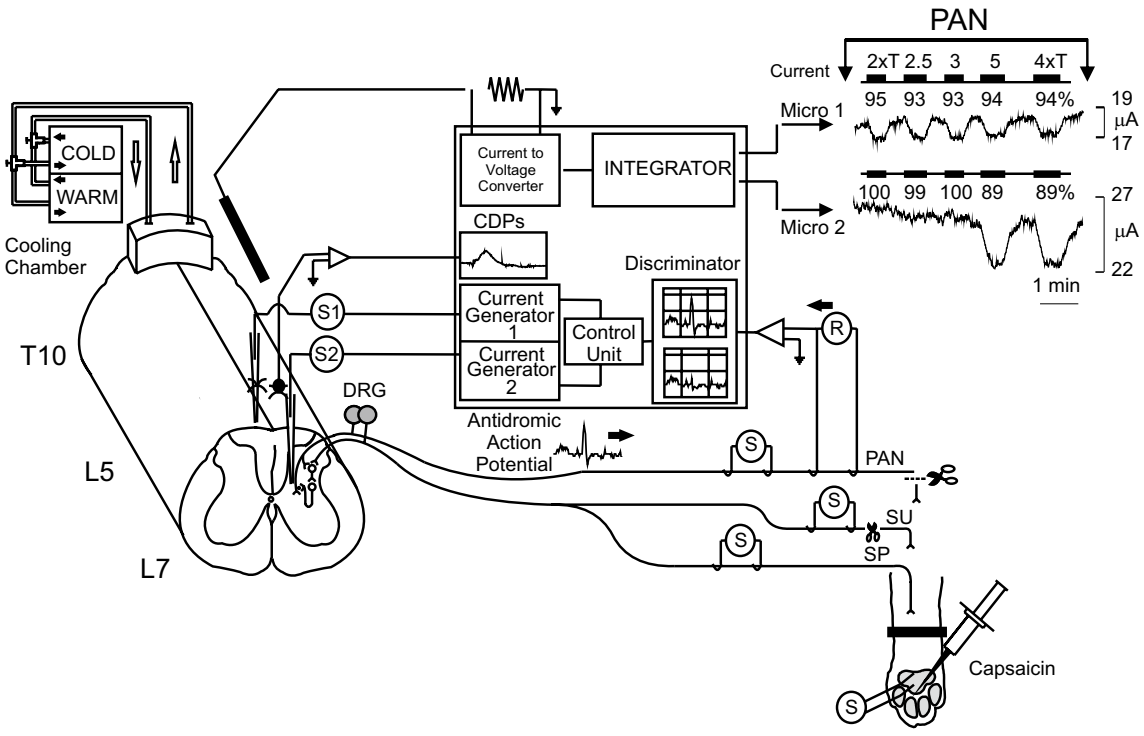


Fig. 1 Schematic diagram of the experimental method. The left posterior articular nerve (PAN) was dissected free, sectioned and its central end mounted on two pairs of hook electrodes, one for stimulation (S) and the other (R) to record the antidromic action potentials elicited in single fibers by intraspinal microstimulation (s1 and s2). Stimulating electrodes were also placed on the SU and SP nerves. Primary afferent depolarization (PAD) and primary afferent hyperpolarization (PAH) of single PAN afferents produced by conditioning stimulation of sensory nerves were inferred from changes in the intraspinal current needed to generate antidromic action potentials with a constant probability (50%). The required current was continuously recorded and stored for subsequent analysis (see Madrid et al. 1979). Changes in the intraspinal threshold were calculated as percentage relative to the threshold of the fiber determined before the conditioning stimulation. Reversible spinal block was made with a silver-plated thermode placed over the surface of the exposed spinal cord at low thoracic level (T10). Capsaicin (30 µl of 1% solution) was injected intradermally into the left plantar footpad. See text and for additional explanations

values maintained until the next cycle to display a continuous recording of the fiber's threshold. These values were digitized and stored to allow subsequent calculations (see below). It is well established that during PAD less current is required for antidromic firing of the afferent fiber. During inhibition of background PAD (PAH), the stimulating current is increased (see Burke and Rudomin 1977; Rudomin and Schmidt 1999).

Mean thresholds were calculated from the digitized data points by setting two cursors in the region where the threshold changes had already attained a steady value (see Rudomin et al. 2004). The percentage threshold changes produced by a given conditioning stimulus were calculated relative to the resting threshold of the fiber.

In this study, we examined the effects of the intradermal injection of capsaicin and of spinal cold block on the intraspinal threshold of the same articular afferent fiber during prolonged time periods, usually between 4 and 12 h ($6.6.0 \pm 1.8$ h).

These were difficult experiments because they required rather stable recording conditions and continuous verification that the excitability tests were indeed performed *on the same* afferent fiber, particularly after the injection of capsaicin and also after spinal block. Here we report data obtained from 32 fibers that we could thoroughly examine for several hours after the intradermal injection of capsaicin. In these experiments we verified that the amplitude, shape and latency of the antidromic potentials recorded in the central end of the PAN remained the same throughout the whole observation period. In general, this was the case after the injection of capsaicin and also during spinal block, but in some cases after several hours of recording, the amplitude of the antidromic response was slightly reduced and the antidromic latency shortened as shown in Figs. 2E, F, 4C,D.

In addition, we verified that the observed changes in the intraspinal threshold of the examined fibers were not due to the blood pressure changes induced by the intradermal injection of capsaicin or during spinal block (see Rudomin et al. 2004).

Peripheral thresholds and conduction velocities

The collision test was made to determine the peripheral electrical threshold of the afferent fiber whose intraspinal threshold was being measured. To this end, a conditioning pulse with graded strengths was applied to PAN through a bipolar electrode placed on the nerve, closer to the spinal cord. The conditioning stimulus was followed by an intraspinal pulse strong enough as to produce in all trials antidromic action potentials of single fibers in the distal end of the sectioned PAN nerve. A sufficiently short conditioning-test stimulus interval (2.5–4.5 ms) was selected to ensure that the test stimulus would be applied during the refractory period that followed activation of the articular afferents by the conditioning stimulus. The strength of the conditioning stimulus was increased until orthodromically conducted spikes evoked by this stimulus collided with the antidromic spikes produced by the test intraspinal stimulus (see Fig. 3E and Rudomin et al. 1986; Quevedo et al. 1995).

The peripheral conduction velocity of the examined afferents was calculated using the antidromic latency from which 0.2 ms was subtracted to account for the latent period of spike generation (see Jankowska and Roberts 1972; Jankowska et al. 1993) and the distance between the intraspinal micropipette and the recording site measured during the experiment (range 15.8–20.5 cm).

Capsaicin administration

Changes in the intraspinal threshold of single articular afferents were recorded before and after a single intradermal injection of capsaicin in the plantar cushion of the left hindlimb (30 µl of 1% solution, in a 10% Tween 80 and 90% saline; see Contreras-Hernández et al. 2018). No further injections were made to avoid desensitization (Sorkin and McAdoo 1993; Sakurada et al. 2005).

Spinal cord cold block

As in previous studies (Quevedo et al. 1993), a silver-plated thermode covered with insulating lacquer was placed over the surface of the exposed spinal cord at low thoracic level (T10). The temperature of the thermode was changed from a warm (37 °C) to a cold (−4 °C) circulating fluid. The thermode had an attached thermocouple that allowed measurement of the temperature at the surface of the cord below the cooling chamber. We used cold blocks lasting 12–30 min (mean 20.8 ± 6.6 min), known from previous studies to produce reversible spinalizations (see Fig. 2B and Laird and Cervero 1990; Cervero et al. 1991; Schaible et al. 1991; Quevedo et al. 1993).

Data analysis

Paired t test and Wilcoxon signed-rank tests. t tests were used to assess the significance between the intraspinal threshold changes of articular afferents produced by autogenic and heterogenic conditioning during different experimental procedures (spinal block, intradermal injection of capsaicin). $p < 0.05$ values were considered as significant.

Similitude tests. This measure is based on the test developed by Bityukov et al. (2016). As in a previous study (see Contreras- Hernández et al. 2018 for details), tests of similarity between paired clusters selected from the whole set of intraspinal threshold changes were made to compare the effects exerted by the intradermal injection of capsaicin

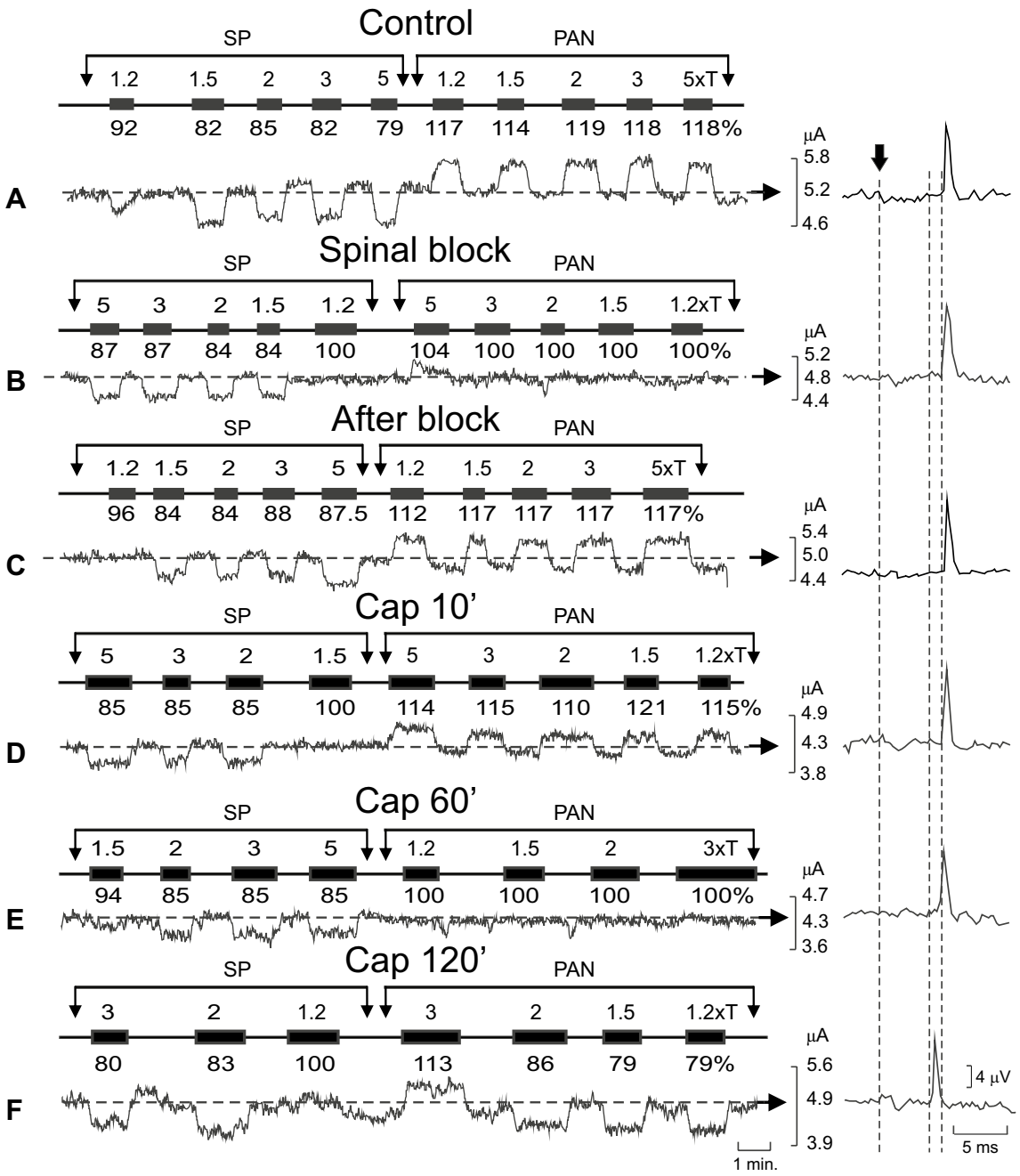


Fig. 2 Intradermal injection of Capsaicin changes autogenic PAH to PAD. **A** Changes in the intraspinal current required to produce antidromic firing of a single PAN afferent produced by conditioning stimulation of PAN and SP nerves with different strengths, as indicated. Note that SP stimulation produced PAD and PAN stimulation produced PAH. **B** The PAH produced by 1.2–3xT PAN stimuli was no longer observed during spinal cold block, in contrast with the slight reduction of the PAD produced by SP stimulation. **C** After removing the spinal block there was a full recovery of the effects produced by PAN and SP stimulation. **D** The PAH and PAD produced by PAN and SP stimulation was still elicited during the first 10 min after the injection of capsaicin. **E** By 60 min after the injection of capsaicin autogenic PAN stimulation no longer produced PAH. **F** 120 min after Capsaicin, the PAN 1.2–2xT PAN stimuli produced PAD instead of PAH. The recordings displayed on the right side of the figure are samples of the averaged antidromic action potentials ($n=5$) elicited in this fiber during each of the different experimental procedures. Spinal location of the excitability testing is shown in Fig. 7D. Further explanations in text

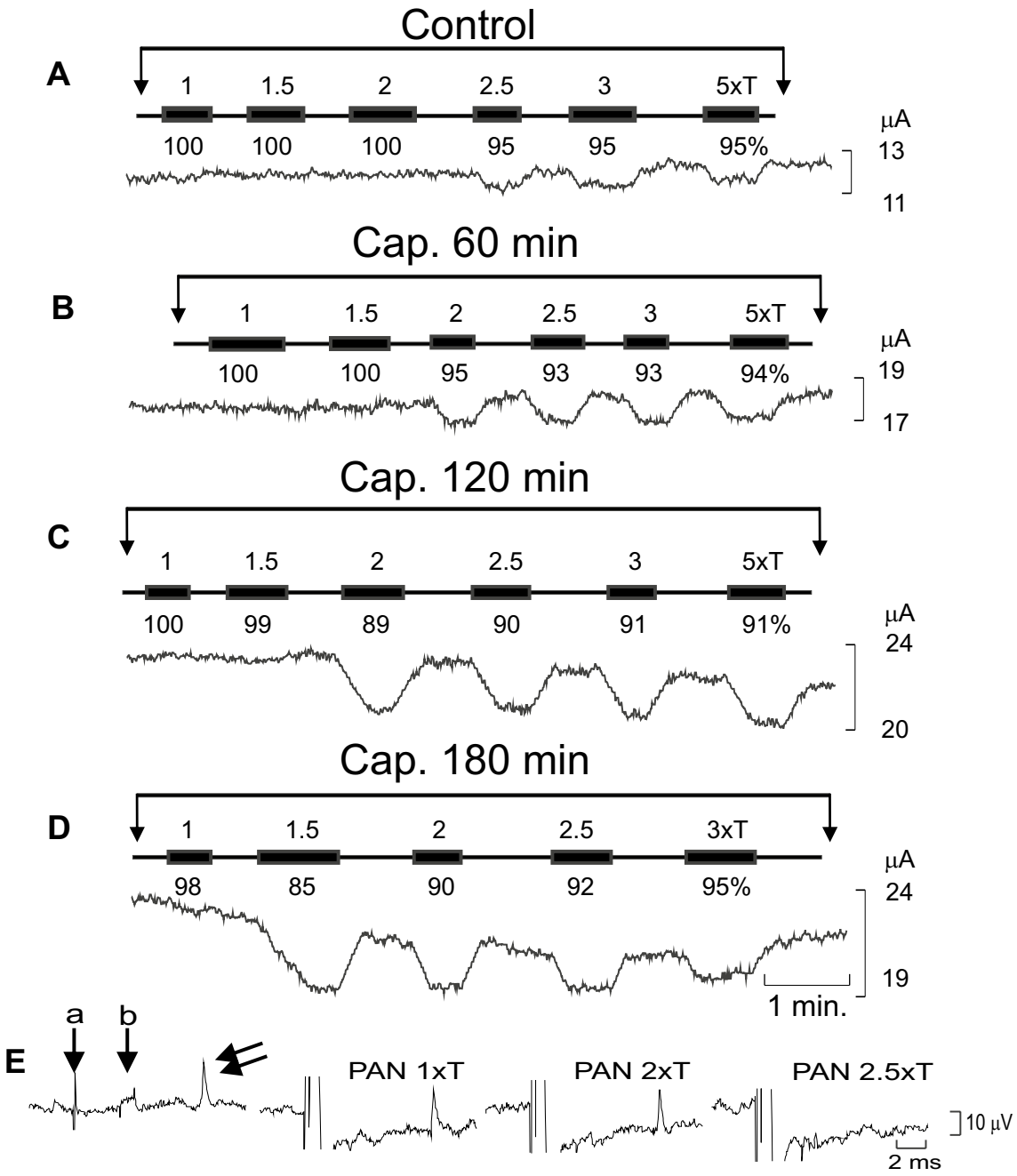


Fig. 3 The intradermic injection of capsaicin increases the autogenic PAD. **A**–**D** Changes in the autogenic PAD produced by stimulation of the PAN with several strengths tested at different times after the intradermic injection of capsaicin, as indicated. Note increased autogenic PAD produced by PAN stimulation applied 120–180 min after capsaicin. **E** Collision tests made to determine the peripheral threshold of the fiber (see Methods). First black arrow (a) points at conditioning PAN stimulus and second arrow (b) at intraspinal test stimulus. Double headed arrow points at antidromic response of the afferent fiber. This fiber had a conduction velocity of 33 m/s Spinal location of excitability testing is shown in Fig. 7D. Further explanations in text

and spinal blocks. We calculated the root mean-square significance (SRMS) between paired clusters. Briefly, RMS ≈ 0 indicates identical sets, RMS ≈ 1 indicates that the two sets are different, but they come from the same population and RMS ≫ 1 indicates that both sets are completely different. This test allows assessment of the similarity in the probability distribution of the intraespinal threshold changes generated in many articular afferents during different experimental procedures.

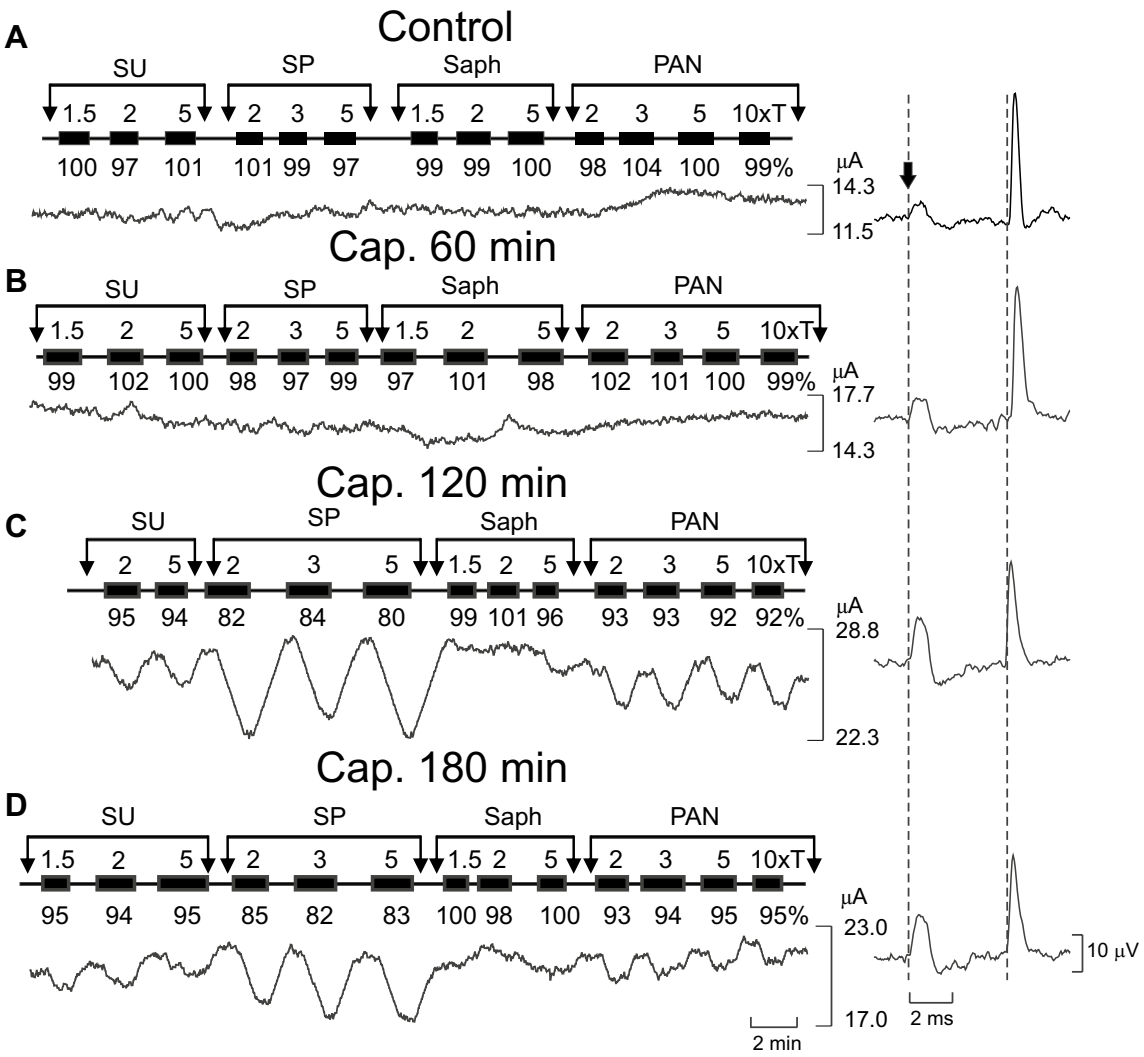


Fig. 4 Emergence of autogenic and heterogenic PAD during the central sensitization induced by the intradermic injection of capsaicin. **A**, **B** conditioning stimulation of PAN and of cutaneous nerves (SP, SU and Saph) had no effect on the intraspinal threshold of a single articular afferent when tested before and 60 min after the injection of capsaicin. **C**, **D** by 120 and 180 min after the injection of capsaicin, SP, SU and PAN stimulation produced a clear PAD. Note that PAN stimulation with intensities below the peripheral threshold of the fiber (4xT) produced autogenic PAD. As in Fig. 2, traces on the right show the antidromic action potentials produced by intraspinal microstimulation. Fiber´s conduction velocity was 39.1 m/s. Spinal location of excitability testing is shown in Fig. 7D. Further explanations in text

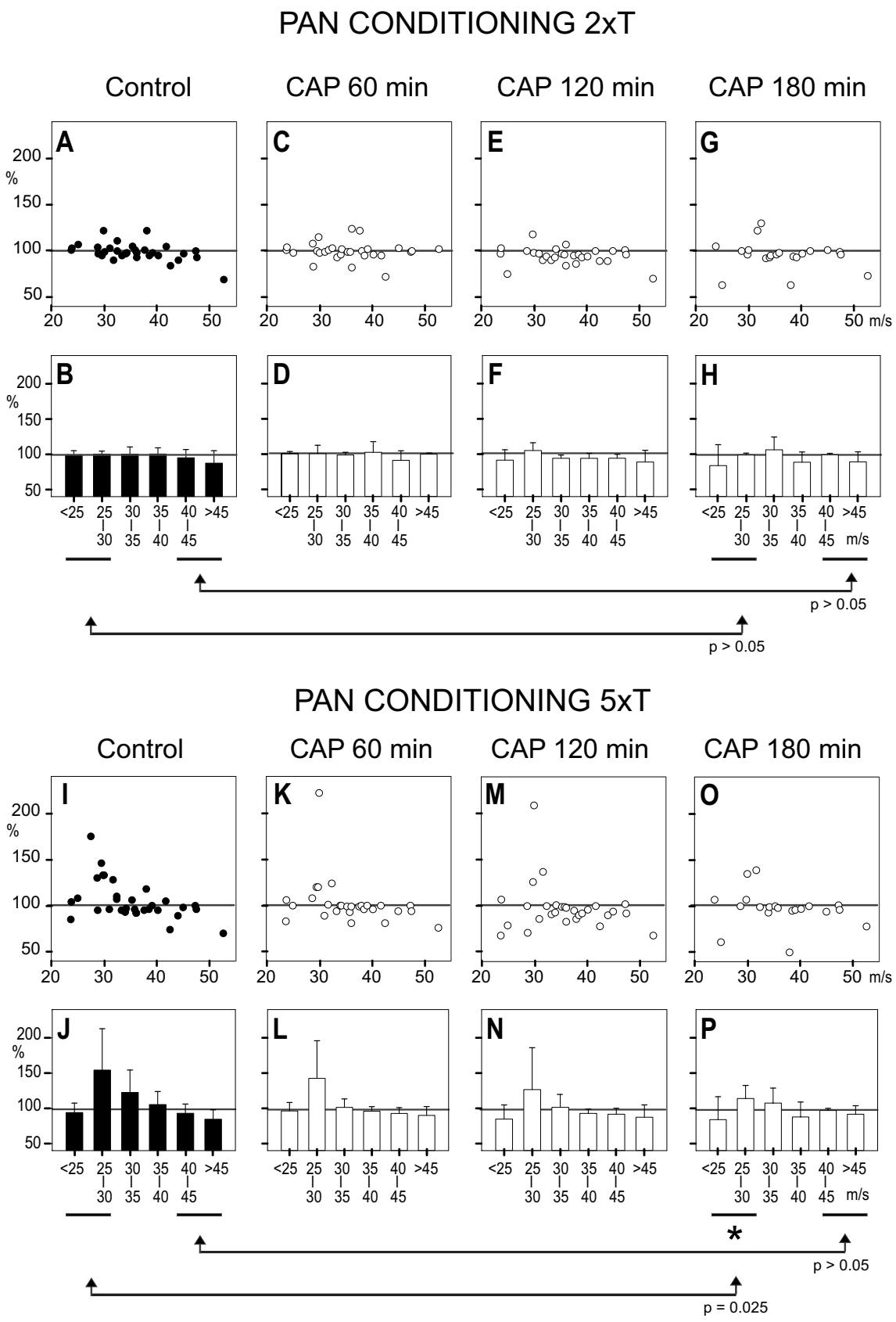
Histology

At the end of the experiment, the animal was euthanized with a pentobarbital overdose and perfused with 10% formalin; the spinal cord was removed, leaving the recording micropipettes in place. After fixation and dehydration, the spinal cord segment containing the recording micropipettes was placed in a solution of methyl salicylate for clearing. Subsequently, the spinal cord was cut transversely to obtain sections containing the recording micropipettes. Location of the excitability testing sites was estimated from the recording depths made during the experiment (Wall and Werman 1976).

Results

Effects of capsaicin on the intraspinal threshold of the joint afferents

The present observations are based on a sample of 32 single articular afferents whose intraspinal threshold could be reliably measured for several hours after the intradermal injection of capsaicin. Our analysis started therefore by comparing effects of capsaicin and spinal block on primary afferent hyperpolarization (PAH) and primary afferent depolarization (PAD) evoked in joint afferents by PAN and SP conditioning stimulation (see below). Only at the


PAN CONDITIONING 2xT
Control
CAP 60 min
CAP 120 min
CAP 180 min
A
C
E
G
B
D
F
H
200
%
150
100
50
20 30 40 50 m/s
<25 25 30 35 40 >45
30 35 40 45 m/s
p > 0.05
p > 0.05
PAN CONDITIONING 5xT
Control
CAP 60 min
CAP 120 min
CAP 180 min
I
K
M
O
J
L
N
P
*
p > 0.05
p = 0.025

◂**Fig. 5** Effects of capsaicin on autogenic PAH and PAD produced by different strengths of PAN conditioning stimulation. **A**–**H** and **I**–**P** Intraspinal threshold changes produced in each of the examined articular afferents by PAN conditioning stimulation with trains of pulses 2xT and 5xT plotted against their conduction velocities. Intraspinal threshold changes expressed as percentage relative to baseline were determined before and after the intradermal injection of capsaicin at the indicated times. The histograms below each panel show the means and SD's of the intraspinal thresholds assembled in different ranges of conduction velocities, as indicated. P values between different sets are shown in brackets. See text for further details

later stages we measured the peripheral thresholds of the examined articular afferents.

Comparison of effects on PAH and PAD

We found that capsaicin had different effects on the analyzed fibers. These effects were related to whether PAD or also PAH were evoked in them by conditioning stimulation of cutaneous and articular nerves. They are illustrated for three fibers in Figs. 2, 3 and 4 and summarized in Figs. 5 and 6.

Figure 2 illustrates the effects of intradermic capsaicin on the PAH and PAD evoked in a single articular afferent by PAN and SP nerve conditioning stimulation. The traces depicted in Fig. 2A show that stimulation of the SP nerve with single pulses of 1.2xT applied 35 ms before the excitability testing pulse reduced the intraspinal threshold of the fiber to 92% of control. That is, it produced PAD. This effect was incremented with stronger stimuli (1.5–5xT) that reduced the intraspinal threshold up to 79%. In contrast, stimulation of the PAN, with relatively weak trains of stimuli (1.2xT) already increased the intraspinal threshold of the articular fiber (to 117%). This effect was slightly incremented with stronger stimuli. We have assumed that the threshold increase produced by PAN autogenic conditioning stimulation was due to inhibition of a tonic PAD. That is, to primary afferent hyperpolarization (PAH; see Burke and Rudomin 1977; Jankowska et al. 1993; Rudomin et al. 1974, 2004, 2007).

Figure 2B illustrates an additional, important and unexpected finding. Namely, that the PAH produced by stimulation of the whole PAN with trains of pulses 1.2–3xT was basically suppressed during a high thoracic spinal cold block, in contrast with the PAD produced by SP conditioning stimulation that was reduced slightly. The PAH produced by PAN autogenic stimulation was resumed when the spinal block was over (Fig. 2C). It thus seems that spinal block reversibly removed the descending action exerted on the pathways involved in the generation of the autogenic PAH.

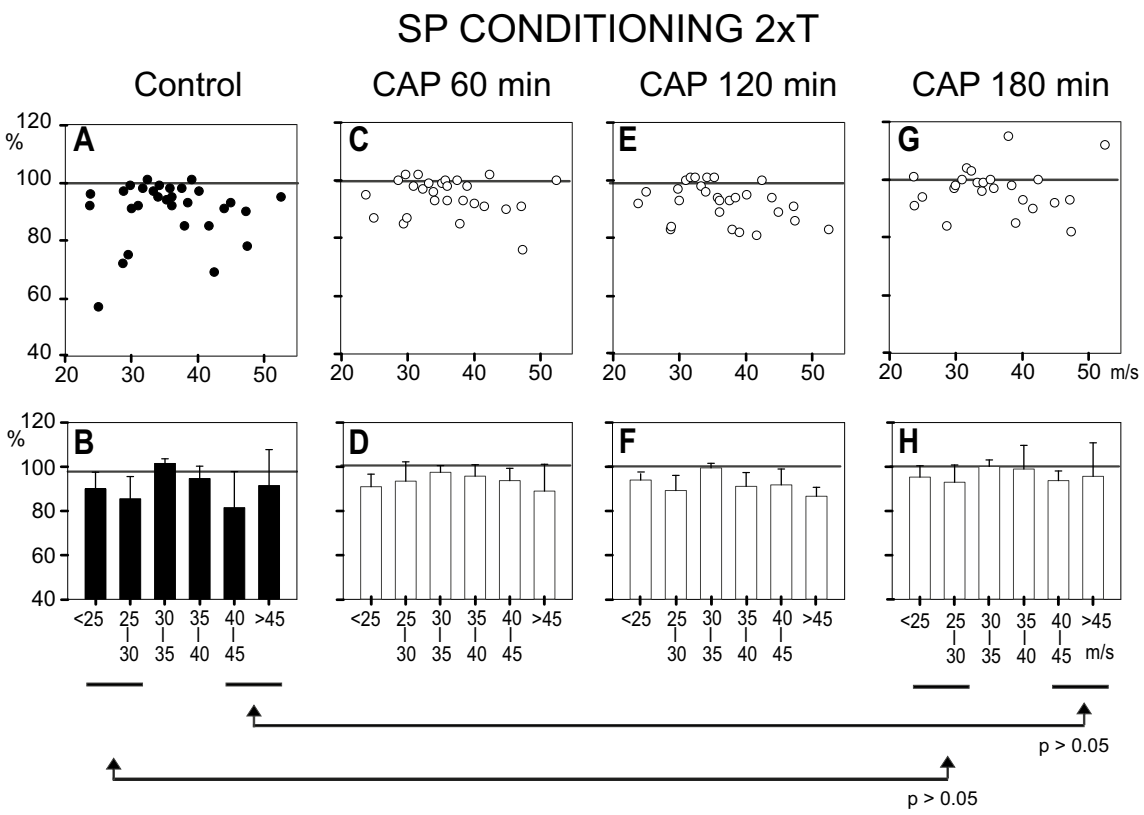
By 10 min after the intradermic injection of capsaicin, stimulation of the PAN still produced PAH, while SP stimulation with pulses above 2xT produced PAD (Fig. 2D). Both effects were of about the same magnitude as those observed before the injection of capsaicin. Yet, 1 h after the injection of capsaicin, the PAH produced by stimulation of the PAN was no longer observed, while stimulation of the SP nerve still reduced the fiber's intraspinal threshold to about the same extent (Fig. 2E), thus resembling the condition attained during spinal block performed before the injection of capsaicin (Fig. 2B).

Stimulation of the SP nerve made 2 h after the injection of capsaicin still produced PAD. However, stimulation of the PAN with the weakest conditioning stimuli (1.2 up to 2.0xT) now produced a clear PAD, while stronger stimuli (3xT) still produced PAH (Fig. 2F). By 3 h after capsaicin, stimulation of the PAN with all strengths produced PAD (not illustrated, but see Fig. 8A that shows intraspinal threshold changes produced in this fiber by PAN conditioning stimulation with different strengths).

As stated in the "Methods", a key question on these observations has been the extent to which the threshold measurements made throughout the whole experiment were performed on the same afferent fiber, particularly after the intradermal injection of capsaicin. The inserts on the right side of Fig. 2 show the antidromic responses produced in this fiber by the intraspinal stimulus made at different times during the experiment. This fiber had a conduction velocity of 38.0 m/s. Under control conditions, the fiber responded to the intraspinal stimulus with an antidromic latency of 5.7 ms that remained the same up to 60 min after the injection of capsaicin (Fig. 2A–E), allowing to interpret effects of capsaicin as evoked at this time on the same fiber. Yet, it was shortened by 0.98 ms 2 h later, at a time when stimulation of the PAN nerve produced PAD (Fig. 2F).

The latency shortening could be due to the increased resting threshold of the fiber (from 4.3 to 4.9 μA), which means that stronger stimuli were required for its antidromic activation. Stronger stimuli could probably activate the myelinated segments of the intraspinal terminals (Lomelí et al. 2000) as well other collaterals of the same afferent fiber (Li et al. 2020) and reduce the latency of the evoked action potentials. The smaller amplitude of the antidromic responses (85% of control) could be attributed to fiber deterioration, most likely because of the damage produced by sectioning its peripheral branch during the dissection, in this case made 12.3 h before. Yet, recruitment of another fiber cannot be excluded.

Figure 3 illustrates data for a single joint afferent in which only PAD was evoked by PAN autogenic stimulation. Under control conditions, stimulation of the PAN with a train of pulses of 2.5xT and above weakly reduced the fiber's intraspinal threshold (to 95% of control, Fig. 3A). One hour after the injection of capsaicin, the resting threshold of the fiber was slightly increased, but even so, the previously ineffective stimuli of 2xT now produced PAD (Fig. 3B). By 2 h after capsaicin, the resting threshold of the fiber was further increased and 2xT PAN stimuli produced larger


SP CONDITIONING 2xT
Control
CAP 60 min
CAP 120 min
CAP 180 min
A
B
C
D
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%
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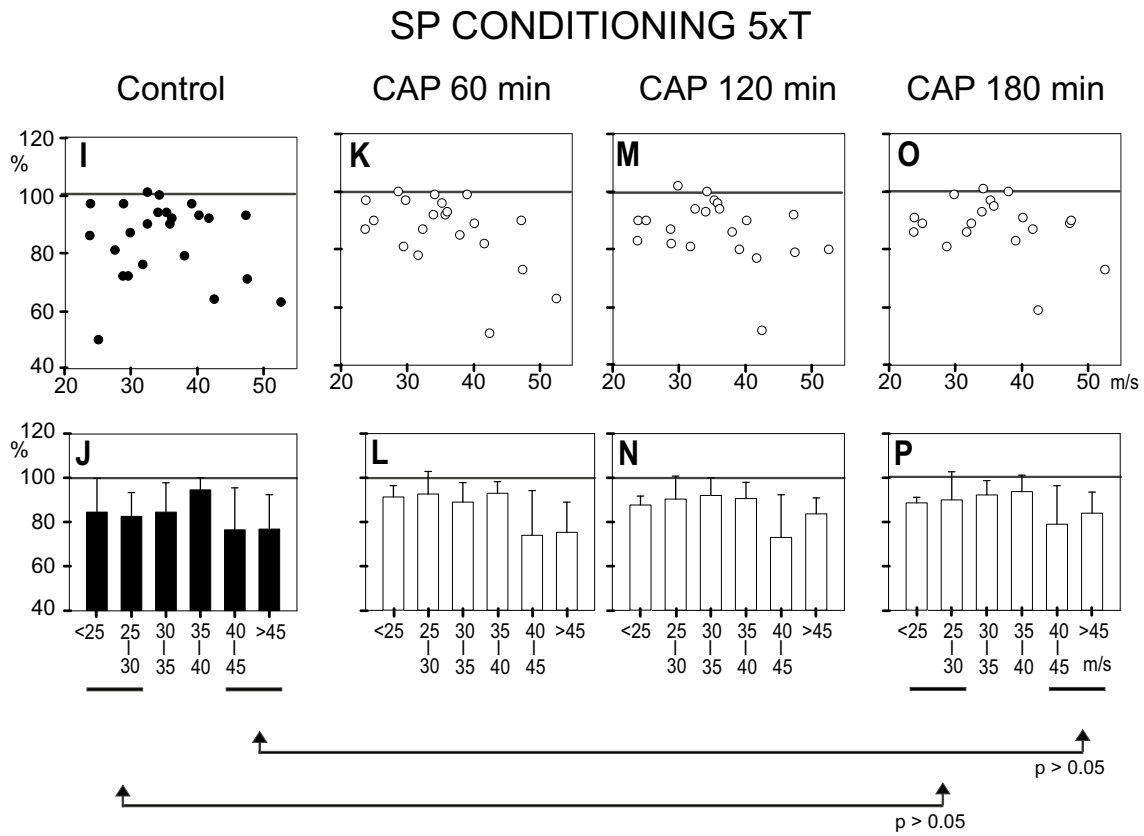
<25
25–30
30–35
35–40
40–45
>45
p > 0.05
p > 0.05


SP CONDITIONING 5xT
Control
CAP 60 min
CAP 120 min
CAP 180 min
I
J
K
L
M
N
O
P
%
m/s
<25
25–30
30–35
35–40
40–45
>45
p > 0.05
p > 0.05

◂**Fig. 6** Capsaicin-induced changes on the PAD elicited by different strengths of SP conditioning stimulation. Same format as that of Fig. 5. **A**–**H** and **I**–**P** intraspinal threshold changes produced in each of the examined articular afferents by SP conditioning stimulation with single pulses 2xT and 5xT, respectively, plotted against their conduction velocity. SP conditioning stimuli were applied before and at different times after the intradermal injection of capsaicin, as indicated. See text for further details

PAD (Fig. 3C) and one hour later also weaker stimuli 1.5xT became effective (Fig. 3D).

One of the questions raised by this series of observations was the extent to which the threshold changes induced by stimulation of the whole PAN were produced by activation of fibers other than those whose threshold was being examined, or else if activation of the examined fiber was a necessary condition to change, because it is known that intrafiber stimulation with high-frequency trains produces a slow post-tetanic hyperpolarization and facilitation of transmitter release (Wall and Johnson 1958; Eccles and Krnjevic 1959).

This question could be easily answered in the case of fibers with peripheral threshold higher than the intensity of stimuli sufficient for evoking PAH or PAD in the tested fiber. As illustrated in Fig. 3E, the intraspinal threshold of this fiber (conduction velocity 33.3 m/s) was above 2xT as the spike induced by intraspinal stimulation was collided by the peripherally evoked spike at 2.5 but not 2xT, while after capsaicin PAN stimuli of 1.5xT and 2xT sufficed to evoke PAD in this fiber. This indicated that the PAD produced with PAN stimuli 1.5 and 2.0xT was due to activation of PAN fibers other than the examined afferent.

Previous studies have shown that in many PAN afferents autogenic stimulation failed to change the intraspinal threshold of the examined fibers even though such stimuli evoked a considerable PAD in cutaneous and muscle afferents (Jankowska et al. 1993; Rudomin and Lomelí 2007). This raised the question on the extent to which the effects of autogenic PAN conditioning stimulation would be modified during the state of central sensitization induced by capsaicin.

Figure 4A, B shows records from a fiber with a conduction velocity of 39.1 m/s and peripheral threshold of 4xT where conditioning stimulation of the SP, SU and Saph nerves and of the PAN (with strengths up to 10xT) had no effect on its intraspinal threshold, both under control conditions and by the first hour after capsaicin. Yet, by 2 h after capsaicin (Fig. 4C), stimulation of the SU and SP nerves with strengths 2–5xT and of the PAN (with strengths 2–10xT), but not of the Saph (strengths up to 5xT) produced a clear PAD. These effects persisted up to 3 h after capsaicin (Fig. 4D). Figure 8G, H shows in addition that the autogenic and heterogenic PAD generated in this fiber after capsaicin was not changed during spinal block.

The recordings displayed on the right side of Fig. 4 show that 60–120 min after the injection of capsaicin, the antidromic latency of the fiber was barely changed while its mean amplitude was reduced (to 80% and 74% by two and three hours after capsaicin, respectively), suggesting that all of the threshold measurements were made on the same fiber.

It should be noted that the resting threshold of the fiber increased gradually after the injection of capsaicin. Similar increases were seen in other fibers (see Fig. 3). This raised the question on the extent to which the gradual increase in the fiber's resting threshold modified the effects produced by the autogenic and heterogenic conditioning stimulation. Although this complicating factor cannot be completely excluded, we have shown previously that expressing the changes as percentage relative to the threshold attained just before the conditioning stimulation provides reliable estimates of the observed changes, despite the shift in baseline threshold (see Eguibar et al. 1997).

Summary of effects of intradermic capsaicin on autogenic and heterogenic PAH and PAD

Based on our original assumption that the descending activity induced by the intradermic injection of capsaicin preferentially facilitates the pathways mediating presynaptic inhibition exerted on the slow conducting, high-threshold articular afferents (see Ramírez-Morales et al. 2019), we examined the extent to which the capsaicin-induced intraspinal threshold changes produced in single articular afferents by autogenic and heterogenic conditioning stimulation were related to their conduction velocity and/or peripheral threshold.

PAN autogenic stimulation

Figure 5A, B shows the intraspinal threshold changes produced in all of the examined afferents by PAN conditioning stimulation with trains of pulses 2xT strength, plotted against their peripheral conduction velocities. It may be seen that under control conditions these conditioning stimuli had basically no effect on the intraspinal threshold of fibers with conduction velocities below 35 m/s, while they slightly reduced the threshold of the faster afferents (above 40 m/s). In contrast, stronger PAN conditioning stimuli (5xT) increased the intraspinal threshold of fibers in the low conduction velocity range (25–35 m/s) while they still produced a slight PAD in faster conducting fibers (above 40 m/s; see Fig. 5I, J).

Quite interestingly, by 1 h after the intradermal injection of capsaicin, the intraspinal threshold changes produced in the articular fibers with the 2xT PAN conditioning stimuli remained basically the same (Fig. 5C, D) but were slightly reduced by 2–3 h after the injection (Fig. 5E–H). On the other hand, the PAH induced by the 5xT autogenic stimulation on the fibers with conduction velocities between 25

and 35 m/s was clearly reduced 60 min after the injection. The reduction of PAH was even more evident two hours later (180 min), as indicated by the relatively low p value ($p < 0.05$). In contrast, the rather small threshold reduction elicited in the fast conducting afferents remained essentially the same after capsaicin even by 3 h after the injection ($p > 0.05$; Fig. 5K–P).

Altogether, these observations indicate that the intradermal injection of capsaicin reduced the PAH elicited in the slow conducting articular afferents by strong (5xT) autogenic conditioning stimulation, basically without affecting the intraspinal threshold of the fast conducting articular afferents.

SP heterogenic stimulation

The effects of capsaicin on the intraspinal threshold changes produced in the articular fibers by SP heterogenic stimulation were different from those elicited by PAN autogenic stimulation. As shown in Fig. 6A, B conditioning SP stimulation with single pulses 2xT mildly reduced the intraspinal threshold of the articular fibers with conduction velocities below 30 m/s and above 40 m/s. Stronger SP conditioning stimulations (5xT) slightly incremented the PAD in both sets of fibers, more in the fastest than in the slowest (Fig. 6I, J).

By 1 h after capsaicin, the PAD produced by SP 2xT conditioning was slightly reduced in both the slow and fast conducting fibers (Fig. 6C, D). In contrast, the PAD produced by the SP 5xT stimulation was clearly reduced in the fibers conducting below 35 m/s and barely affected in the fastest conducting fibers (see Fig. 6K, L), particularly by 2–3 h after the injection of capsaicin (see Fig. 6M–P). Yet, it should be noted that the intraspinal threshold changes displayed by the slow and fast conducting afferents by SP conditioning stimulation before the injection of capsaicin were not significantly different from those attained 120–180 min after the intradermal injection of capsaicin ($p > 0.05$).

Peripheral threshold versus conduction velocity

Altogether, the effects of the intradermal injection of capsaicin on the PAD and PAH of articular afferents elicited by autogenic and heterogenic conditioning stimulations were examined in 32 fibers. As shown in Fig. 7A, in four fibers the PAH induced by autogenic stimulation with strengths 1.5–5xT was suppressed by capsaicin. These fibers had a mean conduction velocity of 31.6 ± 6.1 m/s. In three fibers (mean CV 34.0 ± 3.9 m/s) the PAH changed to PAD while in seven fibers (mean CV 33.5 ± 5.9 m/s) the small PAD was increased after capsaicin and was unaffected in 18 fibers (37.2 ± 7.2 m/s).

In summary, the overall effect of capsaicin was to increase the feed-forward presynaptic inhibition produced by autogenic PAN stimulation, either by reducing the PAH and/or by increasing the PAD elicited in the slow conducting joint afferents.

Figure 7B shows in addition that 2–3 h after the intradermic injection of capsaicin the PAD elicited by SP stimulation with strengths 1.5–5xT was reduced in 12 fibers (mean CV 35.1 ± 8.0 m/s), increased in seven fibers (mean CV 35.3 ± 6.8 m/s) and remained unaffected in 13 fibers (mean CV 35.7 ± 6.1 m/s; not illustrated).

At later stages of this experimental series we used the double shock procedure to measure the peripheral threshold of the PAN afferents (see "Methods" and Fig. 3). We could then examine the relation between the conduction velocity of the examined afferents versus their peripheral threshold. It may be seen in Fig. 7C that the conduction velocity of the examined fibers varied between 23.7 and 52.6 m/s and their peripheral threshold between 1 and 5xT. These distributions were rather similar to those reported for the articular fibers by Jankowska et al. 1993).

The black circles in Fig. 7C show those fibers whose control *autogenic* PAH was reduced or changed to PAD and the gray circles those fibers in which the control autogenic PAD was increased after the intradermal injection of capsaicin. It may be seen that these fibers had conduction velocities below 41.7 m/s (31.7 ± 5.1 m/s) and a peripheral threshold for electrical stimulation that varied through a wide range, from 1 to 5xT (2.75 ± 1.42xT). Those fibers where the effects of PAN stimulation were not affected by injection of capsaicin (empty circles) had somewhat higher conduction velocities, most above 34.2 m/s up to 52.6 m/s (38.3 ± 7.3 m/s). Yet, their peripheral electrical thresholds (2.59 ± 1.44xT) were in the same range as those whose autogenic PAH or PAD was affected by capsaicin (see "Discussion").

Figure 7D shows that most of the excitability tests were made in the dorsal horn (laminae III–V) and that there were no significant differences in the location of the capsaicin-affected and capsaicin-unaffected fibers.

Effects of spinal block on autogenic and heterogenic PAH and PAD

We have shown that during the state of central sensitization produced by the intradermal injection of capsaicin there is a concurrent increase of the descending inhibitory actions exerted on different populations of dorsal horn neurons, among them those responding to PAN stimulation (Ramírez-Morales et al. 2019). These effects were attributed to a descending activation of the spinal pathways mediating presynaptic inhibition of high-threshold nociceptive articular afferents that was exerted without significantly affecting the efficacy of the low-threshold proprioceptive afferents.

It thus seemed of interest to examine the extent to which the intraspinal threshold changes produced in the slow and

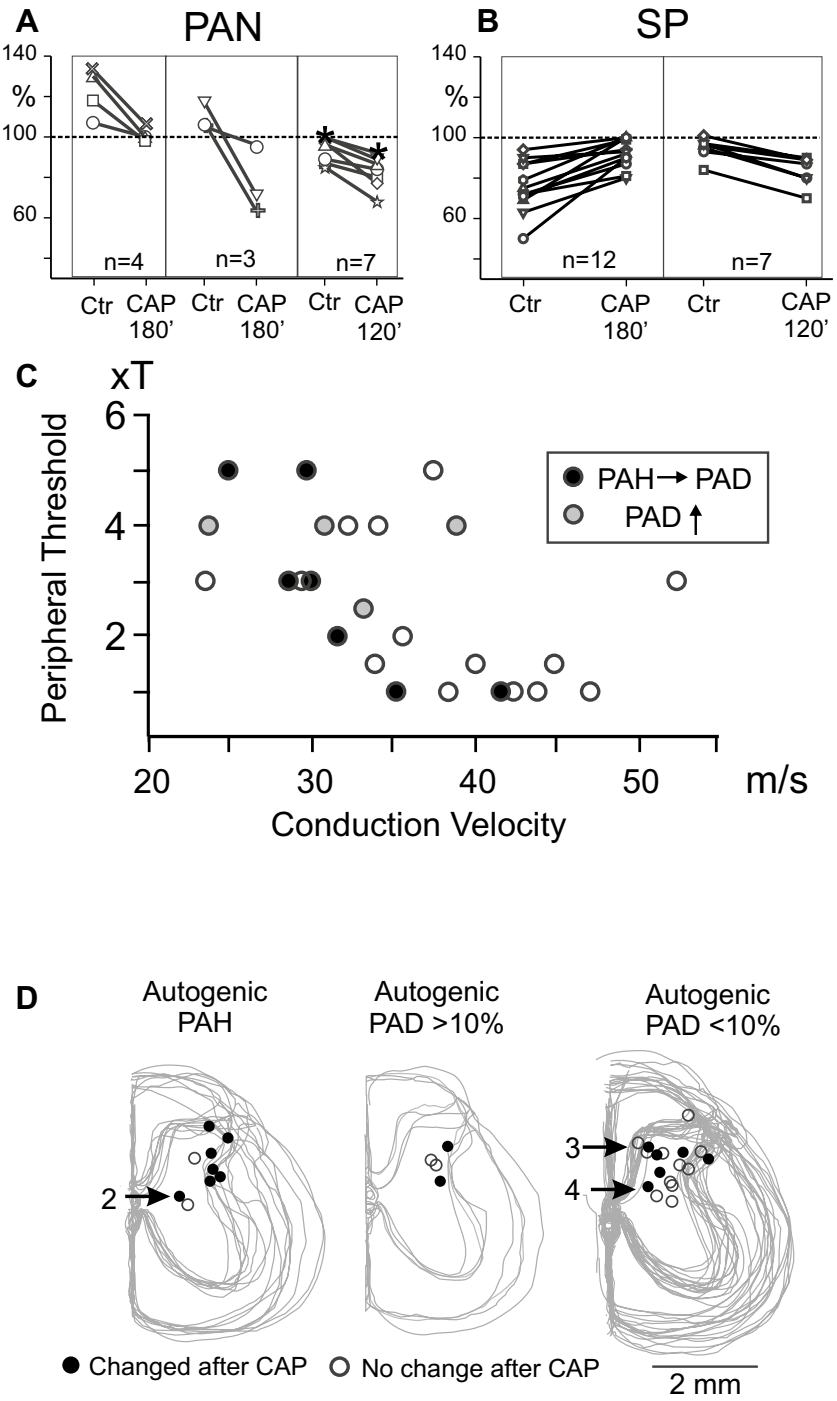


Fig. 7 Conduction velocity, peripheral threshold and intraspinal location of the examined articular fibers. **A**, **B** Summary of intraspinal threshold changes produced by PAN and SP conditioning stimulation delivered 120 and 180 min after the intradermal injection of capsaicin, as indicated. **C** Plot of conduction velocity versus peripheral threshold of the examined fibers. Black circles, fibers whose autogenic PAH was changed to PAD after capsaicin. Gray circles, fibers whose autogenic PAD was increased after capsaicin. Open circles, fibers whose intraspinal thresholds remained unaffected. **D** Spinal location of the excitability tests made on fibers showing increased autogenic PAD, reduced autogenic PAH or no change (< 10%), as indicated. The arrows in Fig. 7D show the site of excitability testing of the fibers illustrated in Figs. 2, 3 and 4. Further explanations in text

fast conducting articular afferents by autogenic and heterogenic conditioning stimulation were affected by the descending modulation activated by nociception.

Figure 8 provides several representative examples of the changes produced by capsaicin and spinal block on the intraspinal threshold of single PAN afferents produced by graded stimulation of the PAN and SP nerves. The data depicted in Fig. 8A, B are of the fiber illustrated in Fig. 2. This fiber had a conduction velocity of 38 m/s. As discussed above, stimulation of the PAN with strengths of 1.2 up to 5xT produced PAH that was reversibly abolished during the first spinal block (first gray bar), was changed to PAD by 120 min after capsaicin, that became largest at 180 min. At that time, a second spinal block suppressed the PAD produced with stimuli up to 3xT (second gray bar). Note that in contrast to the reversibility of the effects induced by the first spinal block made before the injection of capsaicin, there was no apparent reversal of the effects after the second spinal

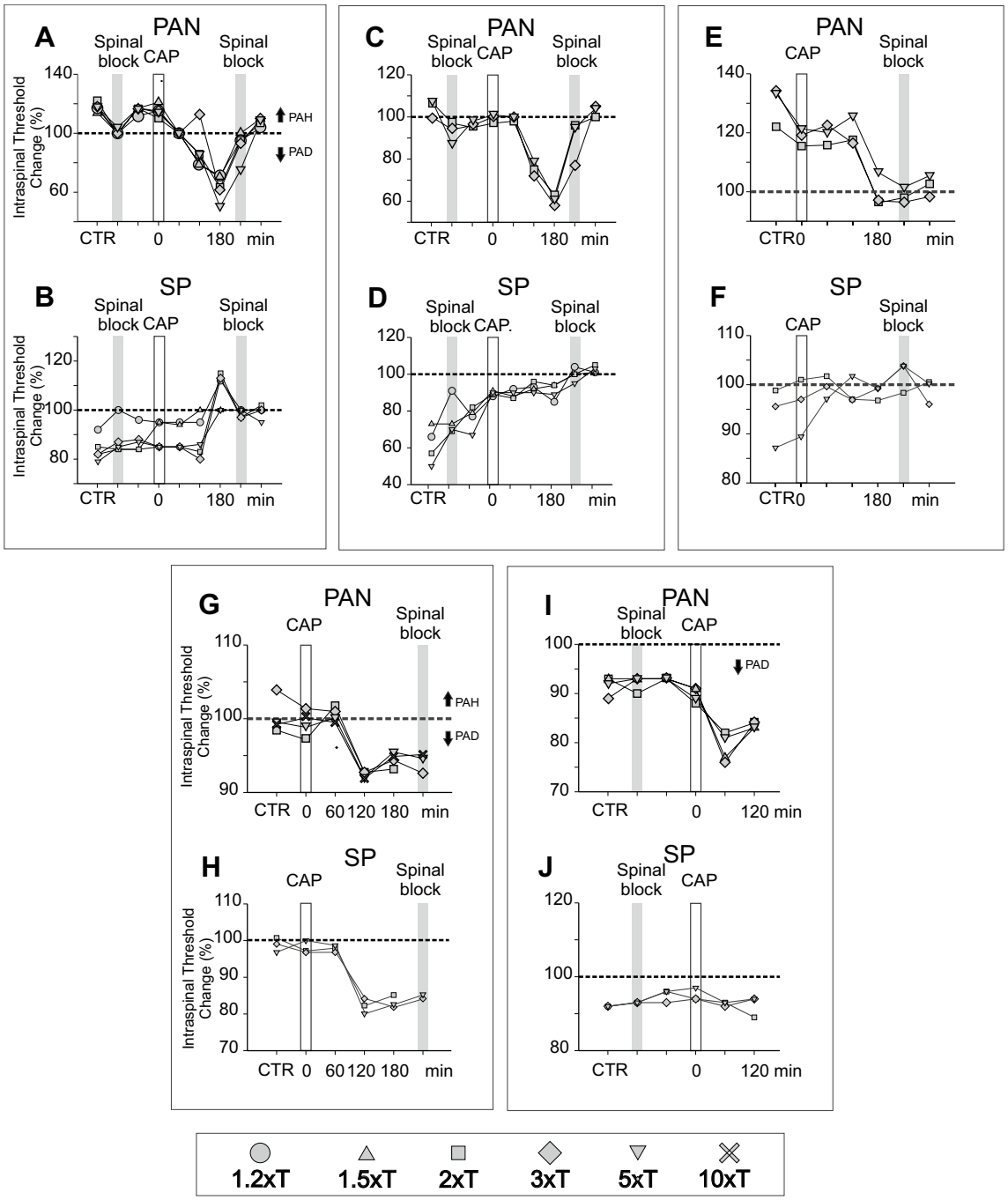


Fig. 8 Effects of spinal block on autogenic and heterogenic PAH and PAD produced in five articular afferents before and after capsaicin. **A–J** Percentage threshold changes relative to control produced by PAN and SP conditioning stimulation with different strengths, as indicated. Gray bars show data obtained during reversible spinal cold block. White bars indicate time of capsaicin injection. Intensity of conditioning stimuli is indicated with different symbols. See text for further details

block. That is, after the block PAN stimulation produced in this fiber no PAD but rather a small PAH, at least for 1 h.

Figure 8B illustrates the intraspinal threshold changes produced in this same fiber by graded conditioning stimulation of the SP nerve. The PAD produced by the weakest stimuli (1.2xT) was abolished during the first spinal block, while the PAD's produced with stronger stimuli were reduced slightly. The effects of SP stimulation on this fiber remained basically the same during the first two hours after the injection of capsaicin and changed one hour later to PAH that was suppressed during the second spinal block and remained so after the block was over.

It is to be noted that in this fiber capsaicin had opposite actions on the intraspinal threshold changes produced by PAN and SP stimulation.

The fiber of Fig. 8C had a conduction velocity of 25 m/s. It showed a mild autogenic PAH that changed to a small PAD during the first spinal block (first gray bar) that was over after removal of the block. 60 min after the injection of capsaicin, PAN stimulation had no effect on the intraspinal threshold of this fiber. Yet, by 120 and 180 min after the injection, PAN stimulation produced a strong PAD that was reduced during a second spinal block. As in the fiber of Fig. 8A, PAD was not recovered after removal of the spinal block, at least for one hour. The data depicted in Fig. 8D show that after capsaicin there was a gradual decrease of the PAD produced by SP stimulation and that spinal block had a rather small effect on these changes.

In Fig. 8E we illustrate the case of a fiber conducting at 29.8 m/s that displayed a considerable autogenic PAH following PAN stimulation that was reduced soon after the injection of capsaicin, kept so for 2 h, almost disappeared by the third hour, and was not restored during the spinal block. The PAD produced in this fiber by SP stimulation was reduced shortly after the injection of capsaicin and remained so thereafter (Fig. 8F).

Figure 8G, H provides a more detailed account of the threshold changes displayed by the fiber whose PAD patterns are illustrated in Fig. 4. In this fiber (CV 39.1 m/s), PAN stimulation had rather small effects before and soon after the injection of capsaicin, but later on produced a stronger PAD that remained so during the spinal block (Fig. 8G). Quite interestingly, following the injection of capsaicin, the PAD produced by SP stimulation was also increased and was also unaffected during spinal block (Fig. 8H).

Finally, in the example of Fig. 8I, the fiber had a conduction velocity of 36.1 m/s and displayed a mild autogenic PAD that was not changed during the spinal block but increased after capsaicin. SP stimulation produced PAD that remained basically unchanged after spinal block and after capsaicin (Fig. 8J).

Summary of changes in PAH and PAD produced by spinal block

The observations displayed in Fig. 8 provide several examples on the effects of spinal block made before and after the intradermal injection of capsaicin. We wondered on the extent to which the observed changes were not only related to the conduction velocity of the articular fibers but if they also depended on whether the conditioning stimuli produced PAH or PAD.

Changes in autogenic PAH and PAD

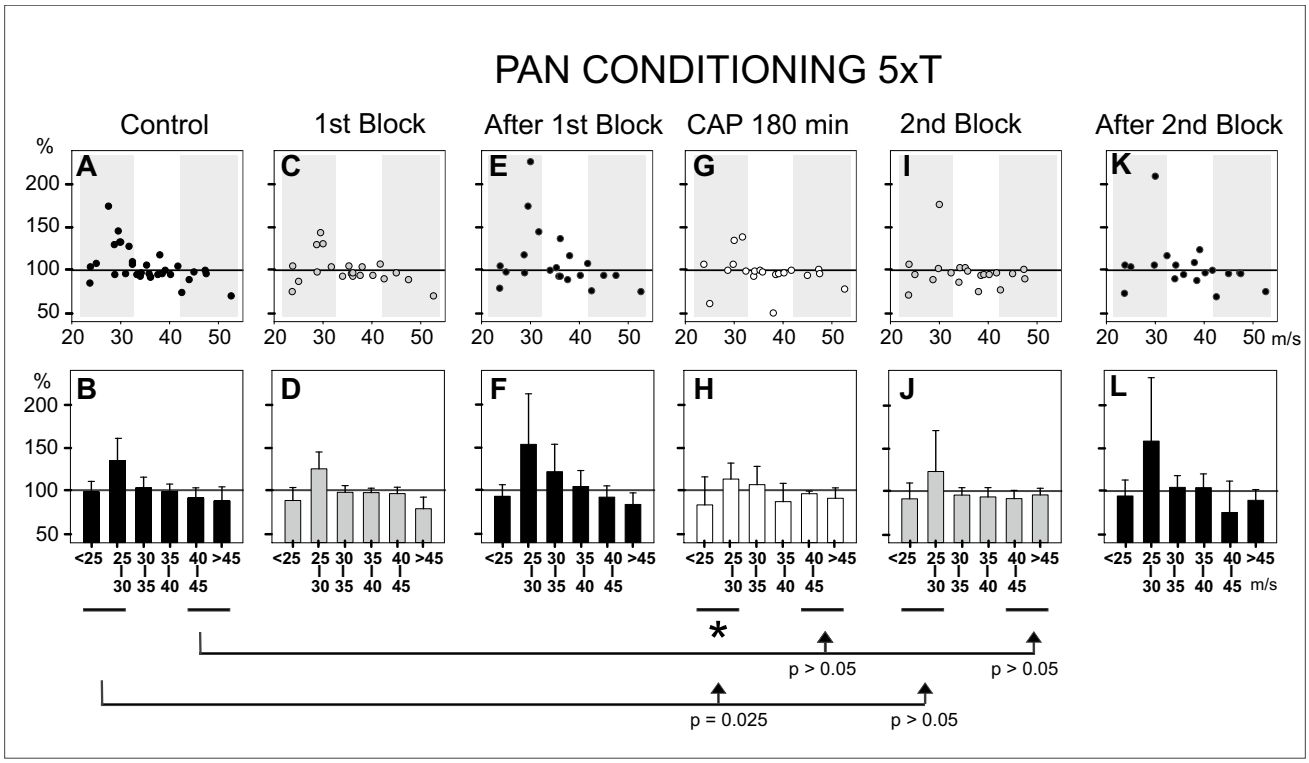
Figure 9A–L shows the effects of spinal cold block on the PAH and PAD produced by autogenic 5xT conditioning stimulation in all of the examined PAN afferents. That is, by conditioning stimuli that produced a clear PAH in slow articular afferents. As in Fig. 5, the intraspinal threshold changes displayed by each afferent were plotted against their conduction velocity. It may be seen that the spinal block made before the injection of capsaicin slightly reduced the PAH displayed by the afferents with conduction velocities between 25 and 30 m/s (Fig. 9A–D) that was followed by an overshoot once the spinal block was removed (Fig. 9E, F). In contrast, the PAD produced by autogenic stimulation in the faster fibers (above 40 m/s) was barely changed during spinalization.

As detailed in Fig. 5, by 3 h after the injection of capsaicin, there was a clear reduction of the PAH elicited in the slow conducting articular fibers (Fig. 9G, H). This effect was partly reverted during a second spinal block (Fig. 9I, J). After the removal of the spinal block, the PAH produced by autogenic stimulation was increased further (Fig. 9K, L). That is, there was no apparent recovery of the effects attained before the spinal block.

It thus seems that before the injection of capsaicin, spinal block had mild effects on the PAH produced by the 5xT autogenic stimulation on the slow conducting afferents. These changes occurred almost without affecting the PAD exerted on the fast conducting fibers. In contrast, the spinal block applied after capsaicin reverted rather clearly the depression of the autogenic PAH, but these changes appeared not to be reversed once the spinal block was removed.

The relatively small effects of spinal block and of capsaicin on the autogenic PAD produced by the 5xT stimuli on the fast (> 40 m/s) conducting afferents illustrated in Fig. 9A–L raised the question on the extent to which these effects depended less on the conduction velocity of the examined fibers but rather on whether the autogenic conditioning stimuli produced PAH or PAD. To this end, as in Rudomin and Lomelí (2007), we ranked the afferent fibers according to the magnitude of the intraspinal threshold changes produced in them by autogenic conditioning stimulations with different strengths. The observed effects were sequentially displayed in descending order. Percentage changes above 100% would indicate PAH and changes below 100%, PAD.

Figure 9M shows the sequentially displayed threshold changes produced by autogenic stimulation with different strengths (1.2-10xT) on the slow conducting (< 30 m/s) articular fibers. It may be seen that these conditioning stimuli produced PAH in most afferents. The open circles in Fig. 9N show that before capsaicin, spinal block reduced the PAH displayed by some of these fibers and as shown in Fig. 9O, this effect was not fully reversible.


PAN CONDITIONING 5xT
Control
1st Block
After 1st Block
CAP 180 min
2nd Block
After 2nd Block
%
A
C
E
G
I
K
B
D
F
H
J
L
200
150
100
50
20 30 40 50
m/s
<25 25 30 30 35 35 40 40 45 >45
*
p > 0.05
p > 0.05
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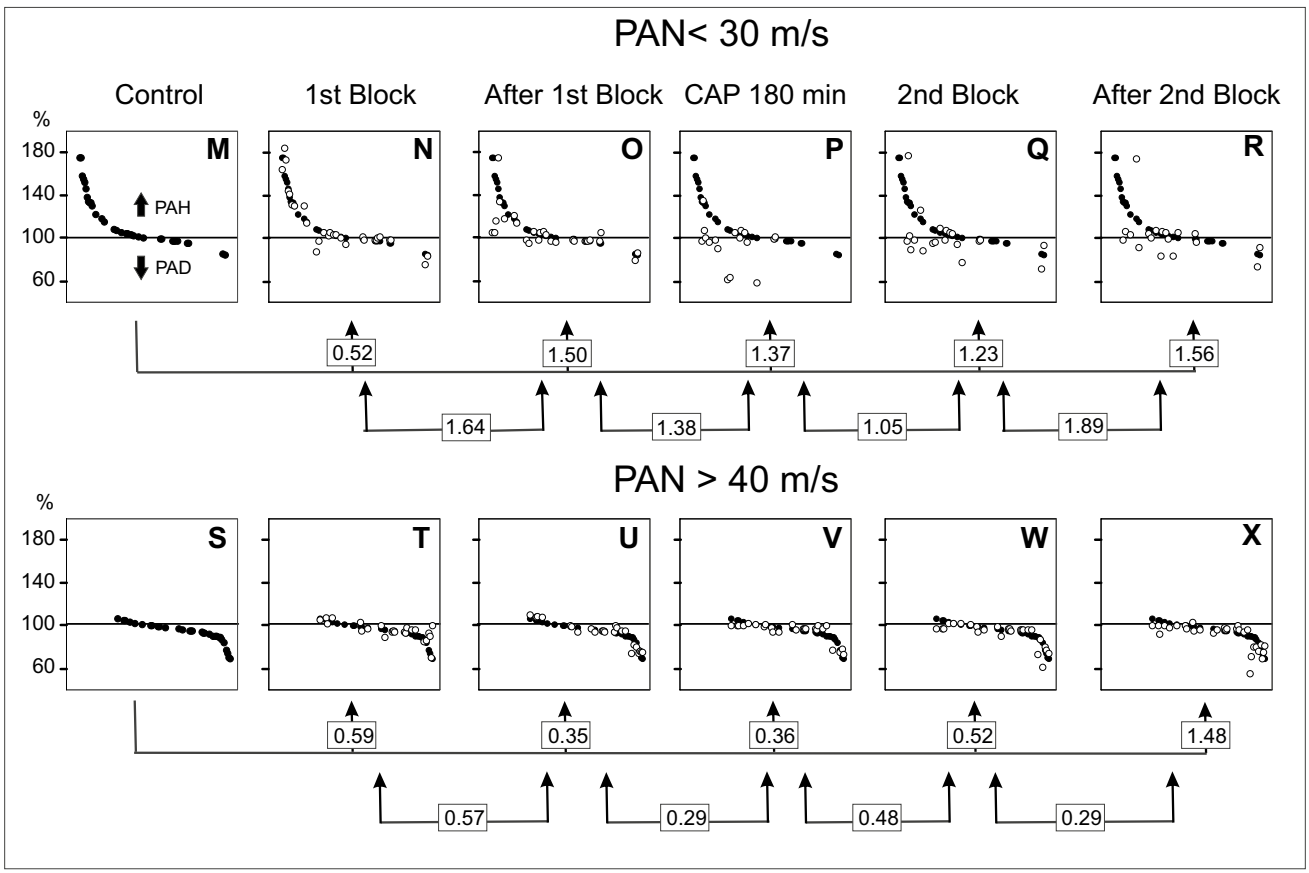
p > 0.05


PAN< 30 m/s
Control
1st Block
After 1st Block
CAP 180 min
2nd Block
After 2nd Block
%
M
N
O
P
Q
R
180
140
100
60
PAH
PAD
0.52
1.50
1.37
1.23
1.56
1.64
1.38
1.05
1.89
PAN > 40 m/s
S
T
U
V
W
X
0.59
0.35
0.36
0.52
1.48
0.57
0.29
0.48
0.29

◂**Fig. 9** Summary of changes in autogenic PAH and PAD produced by spinal block applied before and after the intradermal injection of capsaicin. **A**, **B** Control intraspinal threshold changes produced in single articular fibers by autogenic stimulation with trains of pulses 5xT plotted against their conduction velocity. Note that PAH was generated in fibers with conduction velocities below 30 m/s. **C**, **D** Spinal block reduces the PAH produced by autogenic conditioning stimulation. **E**, **F**, PAH is again increased after the spinal block is over. **G**, **H** The autogenic PAH is depressed 180 min after the intradermal injection of capsaicin. **I**, **J** Spinal block partly reduces the capsaicin-induced depression of PAH. **K**, **L** The effects of spinal block are not reverted following its removal. **M**–**R** and **S**–**X** shows the intraspinal threshold changes produced in slow (< 30 m/s) and fast (> 40 m/s) conducting afferents by autogenic conditioning stimulation sequentially ordered according to their magnitude. **M** and **S** control; **N** and **T** intraspinal threshold changes produced by autogenic conditioning stimulation during spinal block (open circles) together with control distribution (filled circles). **O** and **U**, same but 10–30 min after the spinal block. **P** and **V** intraspinal threshold changes tested 180 min after the intradermal injection of capsaicin. **Q** and **W** same during spinal blocks performed 3.2–4.3 h after the injection of capsaicin. **R** and **X** tests made 18–36 min after the spinal block. The brackets display the significance indices between the different pairs of clusters. Further explanations in text

Figure 9P shows in addition that by 3 h after the intradermic injection of capsaicin, the PAH elicited in several fibers was either suppressed or changed to PAD. A second spinal block applied thereafter reduced the PAD elicited in some of these slow conducting afferents (Fig. 9Q). These changes, albeit small, remained so after the spinal block was over (Fig. 9R).

In contrast with what has been observed for the slow conducting fibers, autogenic stimulation produced PAD in most of the fast conducting afferents (Fig. 9S). Quite interestingly, the intraspinal threshold changes produced by different strengths of autogenic conditioning stimulation on the fast conducting (> 40 m/s) articular afferents remained essentially the same during the spinal blocks and after the intradermic injection of capsaicin (Fig. 9S–X).

As in previous studies (Contreras-Hernández et al. 2018 and Martin et al. 2019) we used the RMS values (henceforth named similarity index or SI) to have an overall appraisal of the statistical significance of the changes produced by autogenic stimulation on the slow and fast conducting fibers during spinal blocks applied before and after the intradermic injection of capsaicin. A similarity index (SI) of 0 would indicate identity between the two sets of data and a SI index of 1, or higher, would indicate that the two sets are different.

It may be seen that after the first spinal block the SI's between the different clusters were consistently higher for the slow conducting fibers (range 1.05–1.89) than for the fast conducting fibers (range 0.29–0.59), suggesting that the major changes produced by spinalization and capsaicin were preferentially exerted on the pathways mediating the PAH elicited in the slow conducting afferents (see "Discussion").

Changes in heterogenic PAD

The effects of spinal block on the intraspinal threshold changes produced by SP conditioning stimulation were not as clear as those produced by PAN autogenic stimulation. Before the injection of capsaicin, spinal block had relatively small effects on the intraspinal threshold changes produced by SP 5xT conditioning stimulation. At most, a slight reduction of the PAD displayed by the slowest conducting fibers (20–25 m/s) and a small increase in the PAD of the fastest conducting fibers (45–50 m/s; Fig. 10A–F). By 180 min after capsaicin the SP-induced PAD was slightly reduced in the slowest as well as in the fast conducting fibers (Fig. 10G, H). The second spinal block further reduced the PAD displayed by the fibers conducting between 25 and 35 m/s and had relatively small effects on fibers conducting above 40 m/s (Fig. 10I, J). These changes were not reversed after the spinal block was over (Fig. 10K, L).

The sequential plots displayed in Fig. 10 M, S show quite clearly that SP conditioning stimulation reduced the intraspinal threshold of both the slow (< 35 m/s) and the fast (> 40 m/s) articular afferents. They also show that spinal blocks applied before the injection of capsaicin increased the PAD displayed by some of the slow and fast conducting afferents, but there was no clear trend in these changes (Fig. 10 N, O, T, U). Yet, by three hours after capsaicin, the most distinctive effect was a clear reduction of the PAD elicited in both the slow and fast conducting afferents (Fig. 10P, V). These effects were not significantly changed during a second spinal block (Fig. 10Q, W) nor after its removal (Fig. 10R, X).

Discussion

Capsaicin-induced changes on autogenic PAH and PAD

We have shown in a recent study that the inflammation produced by the intradermic injection of capsaicin induced a gradual increase in descending inhibition that prevented further facilitation of the responses produced in the dorsal horn by activation of high-threshold ($A\delta$) PAN afferents (Ramírez-Morales et al. 2019). At the same time, only small effects, if any, were found on the responses produced by stimulation of the lower threshold (Aβ) fibers. Since a significant fraction of these afferents converge on common dorsal horn neurons (Basbaum et al. 2009), it was suggested that the capsaicin induced a descending presynaptic inhibitory action that was preferentially exerted on the high-threshold articular afferents, assumed to convey nociceptive information.

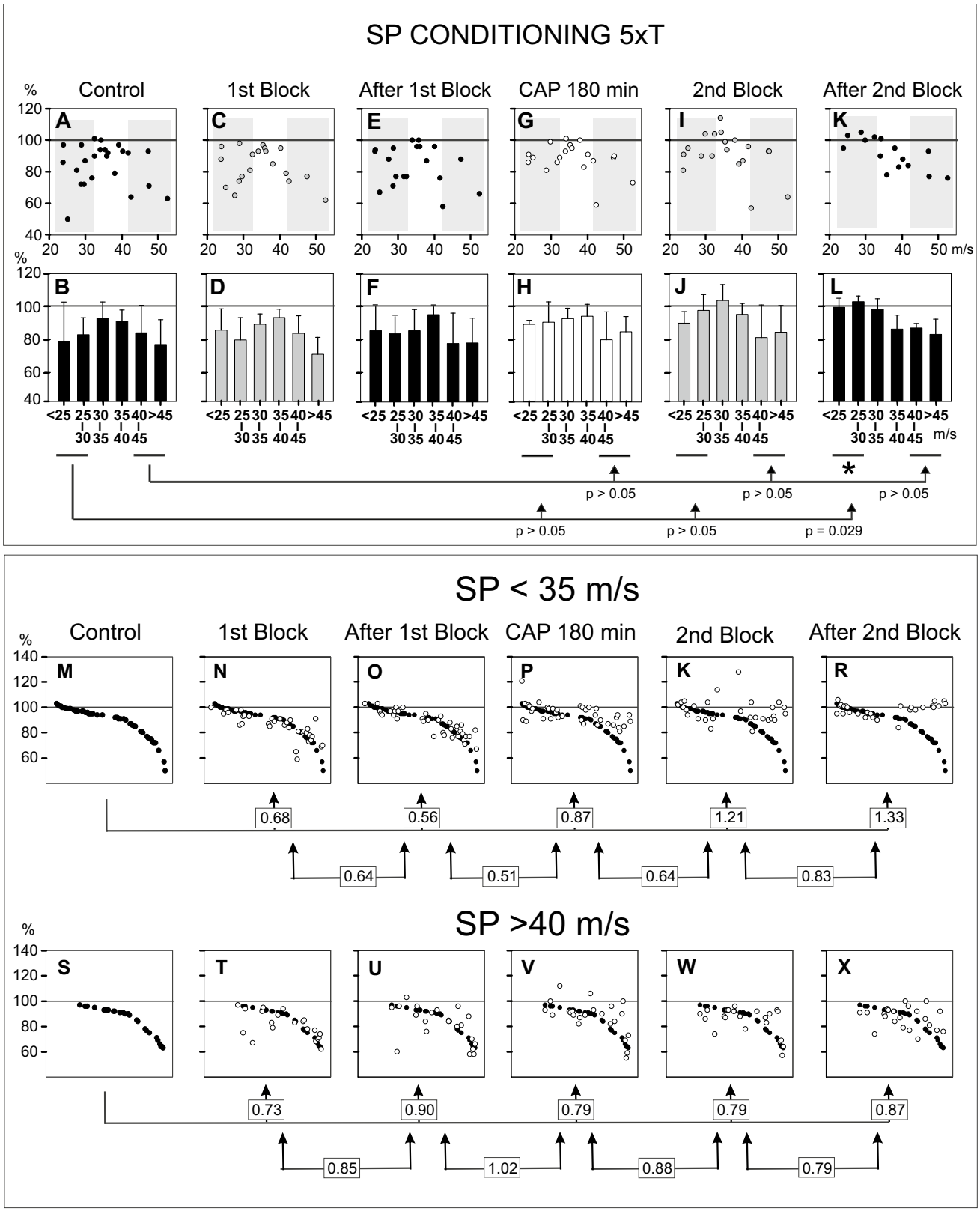


Fig. 10 Summary of effects of spinal block and capsaicin on the heterogenic PAD. Same format as that of Fig. 9 but for the intraspinal threshold changes produced by SP conditioning stimulation. Further explanations in text

The present observations provide direct evidence supporting the selective involvement of presynaptic inhibitory mechanisms of descending origin activated during nociceptive stimulation. Namely, that after the intradermal injection of capsaicin, the autogenic PAH produced in a fair number of slow conducting articular afferents is reduced and gradually changed to autogenic PAD, in contrast with the rather small effects, if any, exerted on the autogenic PAD generated in the fast conducting afferents.

Our observations further indicate that the reduction of the autogenic PAH and its shift to PAD induced by capsaicin is mainly displayed by articular afferents with conduction velocities between 23 and 43 m/s, most of them with peripheral thresholds above 3xT (Fig. 7C). That is, in the *Aδ* range, among them of fibers conveying nociceptive information (Burgess and Clark 1969).

At this point, it should be noted that we found a few fibers with lower peripheral thresholds (< 2xT) whose autogenic PAH was also changed to PAD after capsaicin (Fig. 7C). Since *Aδ* fibers can also have a low peripheral threshold, it is possible that these were also nociceptive afferents. Alternatively, they could be *Aβ* fibers that transmit nociceptive information as it is the case for cutaneous afferents (see Burgess et al. 1968; Djouhri and Lawson 2004). It is not clear, however, if the same condition applies to the low-threshold PAN afferents because, as shown by Burgess and Clark (1969) only "a small fraction (4%) of the PAN afferents responded to bending and twisting procedures considered noxious and these fibers had conduction velocities between 10 and 35 m/s.

Nevertheless, it should be emphasized that neither the fiber's peripheral threshold nor their conduction velocity might fully differentiate nociceptors from proprioceptors (see Jankowska et al. 1993). The main point here is that these two kinds of joint afferents may be subject to a differential presynaptic control.

The degree of inhibition of tonic PAD by homonymous joint afferents expressed in presynaptic afferent hyperpolarization (PAH) might thus also differ in nociceptors and proprioceptors and might to a greater extent depend on descending control (Ramírez-Morales et al. 2019). In fact, it is possible that the differential modulation of the pathways leading to PAD in these afferents during the state of central sensitization initiated by the intradermal injection of capsaicin is mediated by different sets of interneurons, some involving activation of $GABA_A$ receptors and others non-GABAergic NMDA receptors, as suggested by the recent work of Zimmermann et al. (2019). It is also possible that under those conditions, extra-synaptic α_5-$GABA_A$ receptors are also involved in the generation of PAD (see below and Li et al. 2020), as it is the case during chronic pain (Delgado-Lezama et al. 2013; Bravo-Hernández et al. 2016).

Based on the present set of observations, we suggest that the shift of autogenic PAH to PAD induced by prolonged nociceptive stimulation reduces the synaptic efficacy of the high-threshold articular afferents as part of a homeostatic compensatory mechanism that tends to limit the responses of dorsal horn neurons to these and possibly also other nociceptive inputs. This reminds the autogenic PAD generated in Ib afferents during muscle contraction, a situation that has been also interpreted as part of a self-limiting mechanism that filters out Ib inputs on motoneurons and neurons in the dorsal spinocerebellar tract (see Lafleur et al. 1992).

Another relevant finding contributed by the present set of observations is the rather small effect of the intradermal injection of capsaicin on the autogenic PAD elicited in most of the fast conducting articular afferents. As in our previous study (Ramírez-Morales et al. 2019), we have assumed that these afferents transmit proprioceptive information. Preservation of the information carried by these afferents during skin inflammation may be of particular relevance for the proper adjustment of the movement.

It is usually assumed that proprioceptive joint afferents play a relevant role in the information concerning the control of position and limb movement (Baxendale and Ferrell 1985; Ferrel 1980; Ferrell et al. 1985). However, as discussed by Proske and Gandevia (2012), although the receptors involved in control of limb movement proprioception comprise skin, muscle and joint afferents, the information provided by muscle spindles appears to be particular relevant.

At this point it should be mentioned that in the cat, the PAN includes a relatively small number of muscle spindles from the popliteus muscle (about 2%) with conduction velocities between 34 and 107 m/s (Burgess and Clark 1969; see also McIntyre et al. 1978). Hence, it is possible that few group I and group II muscle afferents were included in the present analysis in addition to the proprioceptive joint afferents (see Jankowska et al. 1993 and Riddell et al. 1995). However, as pointed out by Proske and Gandevia (2012), it is not the activity of individual afferents but rather the combined information delivered by the whole ensemble of afferents that provides the required proprioceptive information. As discussed below, a relevant question pertains the role played by the presynaptic control on the information transmitted by the whole ensemble of the afferent fibers.

Capsaicin-induced changes on heterogenic PAD

The few data we have available show that SP conditioning with single pulses, even with the strongest stimuli presently employed (up to 10xT), produced almost no PAH but rather a mild PAD in the slow conducting (25–35 m/s) and a somewhat stronger PAD in the fast conducting articular afferents (above 40 m/s; see Fig. 6A, B, I, J). They also show that the intradermal injection of capsaicin reduced the PAD

elicited in both the slow and in the fastest articular afferents (Fig. 10).

It thus seems reasonable to consider the possibility that the supraspinal control of the PAH and PAD elicited in the articular afferents by heterogenic (SP) conditioning stimulation may not be directly related to the peripheral threshold and/or conduction velocity of the fibers but more to their sensory modality, peripheral location of their sensory fields and on the neuronal populations they activate, as suggested by the observations of Bian et al. (1998) and of Kauppila et al. (1998). Further analysis in functionally identified afferents that remain connected with their peripheral receptors may provide the information required to elucidate this issue.

Supraspinal modulation of autogenic and heterogenic PAH and PAD

As previously shown (Ramirez-Morales et al. 2019), the responses evoked in the dorsal horn by stimulation of high-threshold PAN afferents were increased during a high spinal cold block applied after the intradermal injection of capsaicin. We assumed that this procedure eliminated descending inhibitory influences opposing to further activation of the dorsal horn neurons in response to the nociceptive stimulation.

The present set of observations shows in addition that after the injection of capsaicin, spinalization reduced the autogenic PAH elicited in the slowest PAN afferents without significantly affecting the PAD produced in the fastest fibers (Fig. 9I, J). This suggests that under control conditions the descending modulation, albeit small, was mostly affecting the synaptic efficacy of the slow conducting articular afferents. Quite unexpectedly we found that the effects produced on the autogenic PAH and PAD during the spinal block performed after capsaicin were not reverted after removing the spinal block (see Figs. 8, 9I–L).

We expected that a spinal block applied after the injection of capsaicin would produce rather large changes on the autogenic and heterogenic PAD, because of the suppression by spinalization of the incremented correlation between spontaneous cord dorsum potentials induced by capsaicin (Contreras-Hernández et al. 2018). Yet, it should be noted that in the present series of experiments we examined the effects of spinal block 2.5–4.3 h after the intradermal injection of capsaicin, at times when according to Bonin and De Koninck (2014) the process of memory consolidation produced by intradermal injection of capsaicin would be already established.

Since this process includes a significant reorganization of the functional relations between the different supraspinal nuclei and of their effects on the diverse sets of spinal neurons, it is possible that under those conditions the descending control exerted on the spinal neurons would be attenuated, as suggested by the observations of Danziger et al. (2001). These investigators showed in rats that the tonic descending inhibition of convergent neurons with input from the inflamed ankle was enhanced during the acute stage and then decreased during the chronic stage of mono-arthritis. It is, therefore, possible that a few hours after the injection of capsaicin the spinal circuitry mediating GABAergic presynaptic inhibition would be less responsive to supraspinal control (see Tavares and Lima 2007). In this context, the recent observations of Li et al. (2020) are particularly interesting. They found that in the anesthetized rat, DC epidural polarization produces a long lasting increase in the excitability of the muscle spindle intraspinal collaterals synapsing with motoneurons and with Clarke column neurons. This effect depended on the activation of extra-synaptic $GABA_A$ and $_{\alpha5}GABA_A$ receptors whose action, once initiated, remains for several hours.

To the extent that GABA is released by prolonged nociceptive stimulation and leads to a long lasting depolarization of the spinal collaterals of the articular afferents, it seems reasonable to propose that this process could also contribute to the rather poor reversibility during spinal block of the PAD produced by autogenic and heterogenic stimulation tested several hours after the injection of capsaicin.

In this context our finding that that the autogenic PAH was reduced and even changed to PAD by spinal block, suggests that the recording of negative DRPs by Zimmermann (1965) and by Franz and Iggo (1968) following electrical activation of the C fibers in a cutaneous nerve, instead of the positive DRPs reported by Mendell and Wall (1964), and the PAD elicited in cutaneous afferents by nociceptive skin stimulation with radiant heath (Vyklicky et al. 1969), could be due to the use of low spinal and/or anesthetized preparations, where the background PAD would be low, a situation that would preclude the development of PAH (see Dawson et al. 1970).

Yet, it must be pointed out that PAH was readily observable in the barbiturate anesthetized cats, as reported here and in previous studies (Rudomin and Lomelí 2007), and that the positive DRPs recorded by Mendell and Wall (1964) and by Dawson et al. (1970) were obtained in non-anesthetized, and in high spinal preparations with all peripheral nerves intact. A reasonable explanation would be that in those experiments, the ongoing activity generated by the remaining sensory inputs would still be able to generate a significant background depolarization of the afferent fibers that could be inhibited by stimulation of the C fibers and generate PAH.

The present observations suggest that the shift of PAH to PAD displayed by the high-threshold articular afferents after the intradermal injection of capsaicin is part of a process that prevents an excessive activation of dorsal horn neurons by nociceptive afferents. Based on the available evidence, this process may have several components. One involves

descending mechanisms acting on the spinal presynaptic circuitry that regulates the synaptic efficacy of nociceptive afferents of various origins, among them joint afferents. An increased presynaptic inhibition would restrain the development of a self-potentiating process leading to increased pain and development of allodynia and hyperalgesia (Schaible et al. 1991; Ren and Dubner 1996; Cervero et al. 2003). It is possible that this inhibition involves the diffuse noxious inhibitory control (DNIC; see Le Bars et al. 1979a, b; Danziger et al. 2001; Villanueva 2009; Meléndez-Gallardo and Eblen-Zajjur 2016), whose activation depends on the balance between excitatory and inhibitory descending influences, perhaps mediated by the ON and OFF brainstem neurons (Porreca et al. 2002; Heinricher et al. 1989, 2009).

Quite interestingly, the autogenic PAD elicited in most of the low threshold, fast conducting articular afferents is not significantly changed after the injection of capsaicin. This fits well with the observations of Lin et al. (2000) who found that capsaicin injected intradermally into the plantar skin of the foot increases the dorsal root reflex activity in $A\delta$ and in unmyelinated fibers but not in $A\beta$ fibers (see also Sluka et al. 1995; Willis 1999) and with our previous observations that after capsaicin the N2 dorsal horn field potentials produced by stimulation of the low-threshold articular afferents are not significantly affected during reversible spinalization, in contrast with the facilitation of the N3 responses produced by stimulation of the high-threshold articular afferents (Ramírez-Morales et al. 2019).

Yet, it must be pointed out that under other functional states the synaptic efficacy of articular afferents can be subjected to significant segmental and supraspinal presynaptic control mechanisms as it occurs in cutaneous afferents during active extension or flexion in behaving monkeys (Seki et al. 2003), or during locomotion in spinal cord–hindlimb in vitro preparations (Hayes et al. 2012). In fact, the reduction of the SP-induced PAD observed after the intradermic injection of capsaicin presently described could be envisaged as a mechanism that allows skin afferents to modulate the information carried by the articular fibers, a feature of relevance for the execution of limb movements under normal conditions and also during inflammation, a situation that could partly explain the increased perception of joint pain during skin inflammation (Helliwell and Taylor 2005; Globe et al. 2009).

One interesting feature of the descending presynaptic modulation of sensory information could be the introduction of a frequency code on the afferent fibers via the GABAergic neurons that mediate PAD. This frequency code would be reflected in the activity of the second-order dorsal neurons and could determine, to some extent, the magnitude of their functional coupling with supraspinal structures. We already found that this functional coupling is increased during the state of central sensitization induced by the intradermal injection of capsaicin and is temporarily reversed to the control pre-capsaicin state by systemic lidocaine and suppressed by spinalization (Plamenov et al. 2018). Based on these observations and on previous work (Contreras-Hernández et al. 2018; Martin et al. 2019) we now suggest, as a working hypothesis, that in addition of being a mechanism for selective modulation of the synaptic efficacy and the addressing of information flow to selected neuronal targets as previously suggested (Lomelí et al. 1998; Rudomin and Schmidt 1999), the presynaptic modulation exerted on the afferent fibers could also function as a mechanism that introduces to the intraspinal collaterals of the sensory fibers structured (non-random) correlated influences with a centrally recognizable signature that allows identification of specific functional states of the spinal neuronal networks (see Melzack 1990; Martin et al. 2017).

These concepts complement the views of Proske and Gandevia (2012) pertaining the role of the information transmitted by the whole ensemble of proprioceptive cutaneous muscle and joint afferents in the execution and perception of limb movements, not as a simple addition of the information transmitted by the different sets of afferent fibers but rather a spatial and temporal structured sensory template that can be modified by the descending presynaptic control exerted by the GABAergic neurons, among other possibilities. It is still an open question, the extent to which this scheme is changed during prolonged nociceptive activation that leads to the process of memory reconsolidation and to chronic pain and on how this process is shaped by supraspinal influences and we hope that future research will address these issues.

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