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RESEARCH ARTICLE

Djouhri et al: Impact of inflammation on guinea pig and rat nociceptors

Cutaneous inflammation alters nociceptor electrophysiology in guinea pigs but not rats

Laiche Djouhri[1], Ahmed Eliwa[2], Alghalya Al-Emadi[2], Yehia Y. Hussein[3], Hissa Al-Suwaidi[2], Al-Jouhara Albaloshi[2], Ayman Mustafa[1] and Mohammed Seed Ahmed[1*]

[1]Department of Basic Medical Sciences, College of Medicine, QU Health, Qatar University, Doha, Qatar;

[2]College of Medicine, QU Health, Qatar University, Doha, Qatar;

[3]Department of Orthopedic Surgery, Hamad Medical Corporation, Doha, Qatar.

[*]Correspondence to **Mohammed Seed Ahmed**: mohammed.hamza@qu.edu.qa

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ABSTRACT

Inflammatory pain hypersensitivity is believed to result, in part, from increased excitability of nociceptive dorsal root ganglion (DRG) neurons. We previously demonstrated in guinea pigs that hindlimb inflammation induces electrophysiological changes in these neurons, including faster action potential (AP) and afterhyperpolarization (AHP) kinetics. Given that rats and guinea pigs are distinct species with notable differences in genetic composition and physiology, we hypothesized that cutaneous inflammation would have different effects on the electrophysiological properties of nociceptive DRG neurons in rats—the predominant rodent model for pain research. To test this hypothesis, we performed intracellular voltage recordings from DRG neurons (n = 430) in deeply anesthetized, untreated (control) and CFA (complete Freund's adjuvant)-treated rats and guinea pigs. C-, Aδ-, and Aβ-nociceptors were identified based on their dorsal root conduction velocities (CVs) and responses to natural noxious stimuli. Consistent with our hypothesis, we observed no significant changes in any electrophysiological variables in rat nociceptive neurons four days after CFA-induced hindlimb inflammation. In contrast, guinea pig nociceptors exhibited a significant increase in CV and significant decreases in both AP and AHP durations. The inflammation-induced shortening of absolute and relative refractory periods likely contributes to increased firing frequency in nociceptive nerve fibers, thereby promoting inflammatory pain hypersensitivity. These findings suggest species-specific differences in peripheral neuronal mechanisms underlying inflammatory pain, potentially due to variation in ion channel expression and/or function in DRG neurons between rats and guinea pigs. Given the genetic and metabolic similarities between guinea pigs and humans, further research is warranted to determine whether guinea pigs may serve as a more accurate model of chronic inflammatory pain than rats.

Keywords: tissue inflammation; nociception; inflammatory pain; *in vivo* electrophysiology; nociceptors.

INTRODUCTION

Chronic/persistent inflammatory pain may result from peripheral tissue injury or inflammation. A fundamental feature of this condition is spontaneous/ongoing pain and hypersensitivity to normally nonpainful stimuli (allodynia) and painful stimuli (hyperalgesia) (1, 2). The underlying neuronal mechanisms of chronic inflammatory pain are not fully understood, but preclinical studies using animal models of inflammatory pain suggest that it is partly due to phenotypic changes at more than one level of the nociceptive pathway. Such changes include increased excitability of nociceptive primary afferent neurons (peripheral sensitization) and central neurons (central sensitization) (1, 3-5). It should be noted that central sensitization is believed to be driven partly by input arising from spontaneously active C-fiber afferents (5-7). During chronic inflammation settings, primary afferent neurons become hyperexcitable and start generating spontaneous activity (SA) (abnormal spontaneous nerve impulses/action potentials), the key characteristic of neuronal hyperexcitability. Indeed, using the complete Freund's adjuvant (CFA) model of inflammatory pain, we and others have previously shown that C-and A-fiber dorsal root ganglion (DRG) neurons exhibit SA following persistent cutaneous inflammation (8-11). We have also shown that SA in rat C-nociceptors is correlated with spontaneous pain behavior in the CFA model of inflammatory pain [9].

Other changes in the somata and fibers of DRG neurons innervating inflamed tissue that are believed to contribute to chronic inflammatory pain have also been reported. These include changes in the chemical phenotype in rat Aβ-fibres (12) and in the electrophysiological membrane properties of nociceptive DRG neurons in guinea-pig (8, 13). The electrophysiological changes that we reported previously in guinea pig nociceptors include faster action potential (AP) and afterhyperpolarization (AHP) kinetics as well as increased conduction velocities (8, 13). As we have suggested previously, these changes are likely to increase the ability of nociceptors to carry information to the CNS and thereby contribute to inflammatory pain (8, 13).

Rats and guinea pigs are distinct species with distinct differences in their genetic composition, biology and behaviour (14). Indeed, rats are social creatures that engage in complex social interactions and are known for their agility and climbing skills (15). In contrast, guinea pigs are more submissive, less agile than rodents and exhibit a sedentary disposition (16).Although guinea pigs are in many ways (e.g., genetically and metabolically) more like humans than rats, mice, and even the chimpanzee (17), rats and mice are more often used in biomedical research as animal models of pain than other mammals (see e.g., (18)).

Given that rats are the predominant rodent model for pain research, and that guinea pigs are phylogenetically distinct (19, 20), the aim of this study was to investigate whether the electrophysiological changes observed in guinea pig DRG nociceptors following CFA-induced inflammation also occur in rats. To this end, we made intracellular recordings from somata of lumbar C-, Aδ- and Aβ-fiber DRG neurons in deeply anesthetized normal rats and CFA-treated rats and compared their electrophysiological properties with those in the guinea pig nociceptors four days post CFA.

MATERIALS AND METHODS

Animals

In vivo electrophysiological experiments were conducted on female Wistar rats (180–300 g, Charles River, UK) at the University of Liverpool and female Dunkin-Hartley guinea pigs (180–250 g, Charles River, UK) at Bristol University. The animals were housed in cages in a room with soft bedding and access to food and water *ad libitum*. The room temperature was maintained between 20 and 26˚C while under a 12-hour dark and light cycle. The experimental protocols were approved by the ethical review committees of the two institutions (Bristol University and University of Liverpool, UK) and complied throughout with the UK Home Office Guidelines and UK Animals (Scientific Procedures) Act 1986.

Animal model of chronic inflammatory pain

We used the complete Freund`s adjuvant (CFA) model that involved two intradermal injections of CFA (Sigma, St. Louis, MO) under anaesthesia with 4% halothane, within the cutaneous receptive fields of L4 and L5 DRGs (rats) and L6 and S1 DRGs (guinea pigs). The first injection (100µl) was into the plantar surface of the left hindpaw and the second (100µl) was in the left knee region. This procedure was to induce a unilateral hindlimb inflammation in the whole hindlimb as we described previously (11, 13). CFA, suspended in an oil/saline (1:1) emulsion, was injected at a concentration of 0.5 mg/mL Each 1 mL of CFA solution contains 1 mg of heat-killed and dried Mycobacterium tuberculosis, 0.15 mL of mannide monooleate and 0.85 mL paraffin oil. The control animals received no CFA treatment. It should be noted that the CFA treatment (two intradermal injections) was found to produce an area of localized erythema and edema with 20% mean increase in girth of the ipsilateral foot compared with the contralateral foot [13] and that these symptoms of inflammation were not seen in the hip. Following CFA injection, animals were prepared for *in vivo* electrophysiological recordings as described below.

***In vivo* electrophysiology**

Full details of the surgery and the animal preparation for the *in vivo* electrophysiological recordings from DRG neurons were as we reported previously for the rat (e.g. (11)) and guinea pig (13, 21). Briefly, animals are anaesthetized initially with sodium pentobarbitone (60 mg/kg, i.p.) and kept deeply anaesthetized throughout the experiments with supplementary doses of the anaesthetic (10 mg/kg, i.a.) each hour. Deep anaesthesia is judged by complete absence of limb withdrawal reflex (areflexia). Because the initial dose of the anaesthetic depresses ventilation, a tracheotomy was performed immediately after induction of anaesthesia to allow artificial ventilation and continuous monitoring of end-tidal CO_2. The left jugular vein and carotid artery were cannulated to allow intravenous injections of additional doses of the anaesthetic and to monitor blood pressure respectively.

During the electrophysiological recording, animals were paralyzed with either pancuronium (0.5 mg/kg, i.a.) or gallamine triethiodide (Flaxedil; 2 mg/kg, i.a.). These muscle relaxants were always accompanied by an additional dose of the anaesthetic (10 mg/kg, i.a.) every hour. This dose and frequency of the supplementary anaesthetic was the same before and during paralysis and maintained complete areflexia in the period before paralysis. Core temperature was maintained at 36 ± 0.5°C. Details of exposing and stabilizing the DRGs were as described previously (13). Briefly, following laminectomy, the dorsal root of the DRG under study was cut close to its entry to the spinal cord and laid over a pair of stimulating platinum electrodes. The exposed nervous tissue (DRGs, dorsal root and spinal cord) was protected with liquid paraffin in a large paraffin pool constructed using dental impression material. The recordings were made 4 days after CFA treatment in rats and guinea pigs and in normal rats and guinea of similar age/weight to the CFA-treated animals. Intracellular voltage recordings of somatic action potentials (APs) were made with sharp glass micropipettes filled with 1 M KCl (electrode resistance, 50–120 MΩ) from somata of DRGs (Fig.1). Somatic APs were antidromically evoked by electrical stimulation of the dorsal root using single rectangular pulses (0.03-ms duration for A-fiber units or 0.3 ms for C-fiber units) adjusted to twice threshold voltage for A-fiber units and suprathreshold (x1.5 times threshold) for C-fiber units. Any neurons with high frequency injury discharge were excluded. The temperature in the paraffin pool measured near the DRG being recorded from was 30° to 32°C. APs are recorded on line using a Cambridge Electronic Design (CED, Cambridge, UK) 1401plus interface, and are subsequently analysed offline with CED Spike II program as previously described (11, 13).

Electrophysiological variables measured

A number of electrophysiological variables were measured including the followings (1) membrane potential (Em), (2) AP duration at base (APdB), (3) AP rise time (RT), (4) AP fall time (FT), (5) AP height/amplitude, (6) AP overshoot, (7) AHP depth/amplitude and (8) AHP duration to 80% recovery (AHP80%) (see Fig.1 and Table 1). In addition, conduction velocity (CV) of each neuron is calculated by dividing the conduction distance measured at the end of each experiment (the distance from the stimulating electrode to the recording site in DRG; typically 4 to 7 mm) by the latency from the electrical stimulus artefact to the onset of the evoked AP.

Sensory receptive properties of drg neurons

The sensory receptive properties of DRG neurons were examined with hand-held stimulators and classified as we previously described in the guinea pig (13) and rat (22). Natural noxious mechanical and thermal stimuli were used to identify nociceptive neurons. These include pinch with fine or coarse toothed forceps, sharp objects (e.g. needle) as well as noxious heat (hot water at 50°C or heated glass rod) and noxious cold (<0°C).. The nociceptive neurons included (1) Aβ-, Aδ- and C-fiber high-threshold mechanoreceptive units (HTM) that responded to noxious mechanical but not heat stimuli, and (2) Aδ- and C-fiber units that responded to both noxious mechanical stimuli and also promptly to a single application of noxious heat including: (a) C-fiber units that responded to superficial mechanical stimuli and heat stimuli (C-polymodal nociceptive), (b) C-fiber units that responded to deep mechanical stimuli (probably had dermal receptive fields) and heat stimuli (C- mechano-heat) and (c) Aδ-fiber mechano-heat units with superficial or dermal receptive fields. All these subgroups of nociceptive neurons were clearly recognizable in normal and CFA-treated animals. Unresponsive neurons that could not be excited by any of the aforementioned noxious and non-noxious stimuli were not included in this study. At the end of experiments, animals were sacrificed with an overdose of the anaesthetic.

Neurons were included in the analysis only if their receptive fields were within the inflamed area (the paw and leg but not the hip) and if they had resting membrane potential (Em) of at least -40 mV, an overshooting AP and an AHP, unlike in our previous studies (8, 13) in which we included C-fiber neurons without AHP because the number of subgroups of C-fibre nociceptive units in those studies was too small to make comparison between subgroups. Neurons with cutaneous receptive fields over the hip (outside the inflamed area) were excluded from all analysis. According to their dorsal root CVs, rat neurons were classified as C (≤0.8 m/s), Aδ (1.5 to 6.5 m/s), or Aα/β (>6.5 m/s). This classification is based on compound APs

recorded from L5 and L6 dorsal roots in normal rats with similar age/weight to CFA rats and the under same experimental conditions as those in experiments on CFA rats (23) using the methods that we described previously (8). In the guinea pig, DRG neurons were classified on the basis of their dorsal root CVs as C, Aδ or Aα/β units with C-fiver neurons conducting at < 1.1 m/s, Aδ at 1.1-4.2 m/s, and Aα/β at > 4.2 m/s. This classification is based on recording compound APs from S2 dorsal roots of normal guinea-pigs with similar age and weights to CFA-treated guinea pigs (8). Guinea pig CV values were lower than those in rats, likely due to multiple factors: (1) younger age, (2) lower paraffin pool temperature, (3) inherent differences between dorsal root and peripheral nerve CVs, and (4) inclusion of utilization time, as previously reported [8,13].

Statistical analysis

Most of the data in the control and experimental groups were not normally distributed, and are, therefore, presented as medians and compared with the nonparametric Mann–Whitney U test (Fig. 2-4). The tests were carried out using GraphPad Prism software, version 10 (GraphPad, San Diego, CA). The levels of significance are indicated above the graphs and in Table 1 as follows: *P < 0·05, **P < 0·01, ***P < 0·001. In Table 1, medians are shown, and variability is indicated by the 25% and 75% percentile values for each data set.

RESULTS

Intracellular recordings were made from a total of 225 nociceptive DRG neurons in the rat and 205 in the guinea pig (see Table 1). These were recorded in 23 normal/untreated rats, 20 CFA-treated rats, 24 normal guinea pigs and 21 CFA-treated guinea pigs. Of the rat DRG neurons, 77 were C-fiber nociceptors (43 from CFA rats and 34 normal rats), 82 were Aδ-fiber neurons (58 from normal (untreated) rats and 24 from CFA-treated rats) and the remaining 66 were Aα/β-nociceptors (40 normal and 26 CFA). In the guinea pig, 64 units were C-fiber nociceptors (23 from CFA treated animals and 41 from untreated animals), 69 neurons were Aδ-fiber nociceptors (50 normal and 19 CFA) and 72 neurons were Aα/β-nociceptors (51 normal and 21 CFA).

Hindlimb inflammation induces significant changes in electrophysiological variables in the guinea pig

Comparisons between variables recorded from nociceptive DRG neurons in normal/untreated guinea pigs (no CFA) and CFA-treated guinea pigs (4 days post CFA) are shown in Table 1 and Figures 2-4. As shown in Fig.2 and Table 1 there were, in the C-fiber nociceptive neurons, significantly lower median values in CFA animals compared with no CFA animals in the

following variables: AP duration (Fig.2B), AP rise time (Fig.2C), AP fall time (Fig.2D), and AHP 80% (Fig.2E). C-fiber nociceptors in CFA-treated guinea pigs (but not rats) also showed a significant increase in CV (Fig.2 A and Table 1) compared to untreated guinea pigs. In the Aδ-fiber nociceptors (Fig. 3), there was no significant change in the CV in CFA-treated guinea pigs compared to untreated guinea pigs (Fig.3A), but like C-fiber nociceptors, there were significantly lower median values in the AP duration (Fig.3B), AP rise time (Fig.3C), AP fall time (Fig.3D), and AHP 80% (Fig. 3E) in CFA animals compared with no CFA animals (see also Table 1). As for the Aα/β-fiber nociceptors (Fig. 4), they showed similar changes to Aδ-fiber nociceptors, i.e., the median values of their AP duration (Fig.4B), AP rise time (Fig.4C) and AP fall time (Fig.4D) were significantly lower in CFA animals compared with no CFA animals (see also Table 1). However, the decrease in AHP 80% was not statistically significant (Fig.4E).

Hindlimb inflammation induces no significant changes in electrophysiological variables in the rat

Consistent with our hypothesis and in sharp contrast to the guinea pig, there were no significant changes in any of the variables shown on Table 1 in the CFA rats (4 days after post CFA) compared with no CFA rats (normal/untreated) in any CV group. Indeed, as shown in Table 1 and Fig.2-4, the median values of all the variables measured in CFA rats were not significantly different from those in no CFA rats (normal/untreated). A summary of the changes in electrophysiological properties of nociceptive DRG neurons 4 days after CFA-induced hind limb inflammation in the guinea pig and rat is shown in Table 2. The observed differences in the electrophysiological properties of rat and guinea pig nociceptive DRG neurons tfollowing CFA-induced hind limb inflammation suggest species-specific neuronal mechanisms underlying chronic inflammatory pain.

DISCUSSION

In this study, we used *in vivo* intracellular recordings to determine whether CFA-induced hindlimb inflammation produces electrophysiological changes in rat DRG nociceptors, as we previously found in guinea pigs [8,13]. We performed a side-by-side comparison between the impact of CFA treatment on electrophysiological membrane properties of nociceptive DRG neurons in guinea pigs and rats which are distinct species with distinct differences in their genetic composition. Consistent with our previous findings in the guinea pig, we found significant changes in several variables in guinea pig nociceptors including CV and AP and AHP variables four days after CFA-induced hindlimb inflammation. However, consistent with

our hypothesis, we found no significant changes in any of the variables measured in rat nociceptors suggesting differences in the effects of tissue inflammation on electrophysiological properties of nociceptive DRG neurons in the two species and possibly in the peripheral neuronal mechanisms of inflammatory pain. These apparent differences may arise from differences in ion channel expression and/or function in DRG of the two species. Genetic variations between the two species can result in differential expression of various receptors, ion channels, and signalling molecules involved in nociception.

The inflammation-induced changes in the AP and AHP variables in guinea pig nociceptive DRG neurons are likely to be due to changes in expression and/or biophysical properties of several ion channels including the Na^+ channel ($Na_v1.8$) that underlies AP rise time and overshoot in most nociceptive afferents (24) and Ca^{2+}-dependent K^+ channels and delayed-rectifier type of K^+ channels that respectively mediate AHP and AP repolarization in sensory neurons (see (25)). Several studies suggest that $Na_v1.8$ plays a critical role in inflammation-induced hyperexcitability of afferent sensory neurons and in inflammatory pain. For example, studies using inflamed hind paw models, have shown an upregulation in $Na_v1.8$ expression and the associated slowly inactivating TTX-R current in DRG neurons (26), and an increase in $Na_v1.8$ immunoreactivity, especially in unmyelinated axons (27). Given that this inflammation-induced increase in expression of $Na_v1.8$ channels and TTX-R occurred in rat DRG neurons, our findings of no significant change in AP and AHP variables in this species after CFA-induced inflammation were unexpected. The marked difference that we found in the impact of cutaneous inflammation on the electrophysiological properties of DRG neurons in guinea pigs and rats may reflect differences between the two species in the expression and/or function of the aforementioned channels and other ion channels that are involved in regulating AP and AHP variables in DRG neurons as reported previously for CNS neurons (see below).

To the best of our knowledge, this is the first study to report species differences in the impact of cutaneous inflammation on the electrophysiological properties of DRG neurons. However, species differences in the electrophysiological properties of CNS neurons have been reported previously. For example, marked differences in electrophysiological properties between neurones of the dorsal motor nucleus of the vagus (DMV) in rat and guinea pig have been described (28). These include: (1) larger (higher amplitude) and longer (broader) APs in guinea pig neurons suggesting more Ca^{2+} entry during AP (28), (2) longer AHP durations in guinea pig neurones which would contribute to the slower repetitive firing seen in these neurons (28), (3) two Ca^{2+} -activated K^+ currents ($Gk_{ca,1}$ and $Gk_{ca,2}$, see (29)) in most guinea pig neurones,

but only the $Gk_{ca,1}$ (apamin-sensitive) in the rat neurons, (4) a larger inward rectifier in guinea pig neurones than in that in the rat, and (5) a larger *Ih* in guinea pig DMV neurons that those in the rat neurons. Another *in vitro* electrophysiological study that used whole-cell recordings from central amygdala neurons (30) showed that most central medial and lateral neurons in guinea pigs displayed an outward rectification current that delayed firing onset in response to depolarizing current pulses, whereas these so called late-firing neurons were rare in the rat central nucleus.

It is noteworthy that other differences between guinea pigs and rats have been reported previously including marked differences in the cytochemical properties of their DRG neurons (31). Indeed, guinea pigs have been shown to have significantly higher levels of the neurotransmitter substance P (SP) in the DRG, the dorsal roots and dorsal spinal cord. Guinea pigs have also been shown to have 100-500 fold higher affinities for CP-96,345100, a selective antagonist of SP preferred receptor neurokinin-1 (32). It should be noted that SP (pain neurotransmitter) is found in both peripheral and central terminals of C-fiber nociceptors, is involved in pain transmission and neurogenic inflammation, and its levels are increased during inflammation settings and are associated with heightened pain sensitivity (for reviews see e.g. (33, 34)). Species differences in expression of P2X5 receptors (a subtype of ATP receptors), another receptor type that is also associated with pain signal transmission from the periphery to the spinal cord (35) have been reported. For example, an immunohistochemical study that examined expression of P2X5 receptors in DRG in several mammalian species including rat and guinea pig (36) showed that levels of P2X5 receptors in guinea pig DRG are higher than those in rat DRG.

As already noted, rats and mice are more often used in biomedical research as animal models of pain than other mammals (see e.g., (18)). This is because their genomic, proteomic and metabolomic profiles and their system functions and behavior are better known than other species, and because they are believed to share a closer evolutionary linkage to humans than other non-primate mammals. Furthermore, rats are more widely used in pain research than guinea pigs because of several scientific and practical reasons including: (1) rats exhibit clearer and more quantifiable pain-related behaviors (e.g. licking and guarding) than guinea rats, (2) guinea pigs require specialized care and are more prone to stress, which can confound pain studies and (3) more immunohistochemical and genetic tools are available for rats than for guinea pigs. However, we previously used guinea pigs (8, 13) because, as already noted (see Introduction), they are in many ways more like humans than rats, mice, and even the

chimpanzee. For example, the immune system (37), and the fetal development timing (38) of guinea pigs are similar to humans and different from rats. Metabolically, guinea pigs are also more like humans than rats as they possess cholesteryl ester transfer protein, lipoprotein lipase, and lecithin-cholesterol acyltransferase (39, 40). Furthermore together with primates, guinea pigs are the only laboratory animals with a dietary vitamin C requirement. In contrast, rats do not have the plasma cholesteryl ester transfer protein (40). A marked difference between the rat and guinea in some aspects of the histamine system in the brain including the regional distributions of histamine H1-receptors has also been reported (41). Unlike rats, guinea pigs are 'precocial', i.e. they are born with eyes open with a relatively advanced development of the brain. Indeed, the guinea pig brains are very similar to human's brains at many levels including the Circle of Willis (42). The main strengths of the current study include: (a) the electrophysiological recording of the DRG neurons *in vivo*, i.e., in their natural environment, which is complex and changes with time during inflammatory pain settings in a way that is not fully understood and which, therefore, could not be mimicked *in vitro,* (b) physiological identification of nociceptors, i.e. identification of their receptive properties which is not possible *in vitro* and (c) the large sample size. However, one limitation of our study is the use of female rats only and the electrophysiological recording at only one time point after CFA treatment (day 4 post CFA). Although sex differences are a major area of interest in chronic pain research and there is recent evidence for sex differences in the electrophysiological properties of human DRG neurons (43), this is beyond the scope of the present study. It is noteworthy that we used female animals for practical reasons as we found that performing laminectomy is easier on female than male animals presumably because of their softer bones.

CONCLUSION

Our findings of significant changes in the electrophysiological properties of nociceptive DRG neurons in the guinea pig but not rat following hindlimb inflammation may arise from differences in ion channel expression and/or function in these two species. As we have suggested previously (8, 13), the inflammation-induced decreases in AP and AHP durations (shortening of the AP absolute and relative refractory periods) would result in an increase in the firing frequency of nociceptive nerve fibers, and thereby contribute to pain hypersensitivity. In other words, the inflammation-induced changes are likely to increase the ability of nociceptors to carry information to the CNS and thereby contribute to inflammatory pain hypersensitivity in the guinea pig. Our results suggest species-specific differences in the peripheral neuronal mechanisms of inflammatory pain. However, further comparative studies

are clearly needed to better understand species-specific differences and similarities that would help in the choice of the appropriate species and experimental models of chronic pain to improve the translation of animal research to patients.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Impact of CFA-induced hind limb inflammation on electrophysiological properties of nociceptive DRG neurons in the guinea pig and rat

Animal/ CV group	Animal group	N	CV m/s	Em - (mV)	AP Height (mV)	AP Overshoot (mV)	AP duration at base (ms)	Rise Time (ms)	Fall Time (ms)	AHP depth (mV)	AHP duration 80% (ms)
Rat C-fiber	Normal	**34**	0.4 (0.4-0.6)	54 (61-48)	76 (69-83)	23 (11-28)	5.3 (3.9-6.9)	1.9 (1.4-2.6)	3.3 (2.2-4.7)	7 (3.5-9)	17 (10-28)
	CFA	**43**	0.4 (0.4-0.5)	52 (61-47)	79 (71-91)	27 (20-35)	5.6 (4.0-7.6)	2.0 (1.5-2.7)	3.4 (2.4-4.6)	7 (4.8-11)	24 (15-31)
Guinea Pig C-fiber	Normal	**41**	0.4 (0.3-0.5)	46 (51-42)	67 (60-73)	19 (14-26)	5.0 (3.7-6.1)	2.1 (1.7-2.8)	2.8 (2.0-3.3)	8 (5.1-12)	18 (13-28)
	CFA	**23**	0.4 (0.3-0.7) *	48 (52-42)	72 (62-80)	21 (18-33)	3.7 (3.3-5.0) **	1.8 (1.6-2.1) *	1.9 (1.7-2.9) **	10 (8.1-12	12 (10-20) *
Rat Aδ-fiber	Normal	**58**	5.0 (4-6)	45 (55-45)	68 (59-81)	19 (9-30)	2.6 (2.1-3.2)	1.0 (0.9-1.4)	1.5 (1.2-1.9)	9 (6.5-10)	16 (7-61)
	CFA	**24**	5.0 (4-6)	54 (62-47)	77 (69-85)	21 (13-23)	2.6 (1.9-3.1)	1.1 (0.8-1.9)	1.5 (1.2-1.9)	8 (4.8-11)	26 (10-55)
Guinea Pig	Normal	**50**	3.0 (2-4)	49 (53-44)	72 (63-77)	20 (15-26)	2.8 (2.3-3.3)	1.2 (0.9-1.4)	1.5 (1.3-1.9)	9 (4.7-12)	14 (10-22)

Aδ-fiber	CFA	**19**	2.9 (2-4)	50 (56-42)	68 (54-75)	25 (11-30)	2.2 (1.7-2.4) **	0.9 (0.6-1.1) *	1.2 (1.1-1.4) **	8 (6.0-12)	11 (6.0-15) *
Rat Aα/β-fiber	Normal	**40**	11 (8-15)	56 (60-49)	71 (61-82)	16 (9.0-24)	1.6 (1.3-2.2)	0.7 (0.5-1.0)	0.9 (0.8-1.1)	9 (7.1-10)	15 (8.0-37)
	CFA	**26**	10 (8-15)	54 (63-49)	72 (64-80)	15 (5.0-28)	1.5 (1.3-2.0)	0.6 (0.5-0.9)	0.8 (0.7-1.1)	8 (6.5-12)	11 (5.0-30)
Guinea Pig Aα/β-fiber	Normal	**51**	6.0 (4.9-8)	49 (55-44)	72 (61-76)	20 (11-26)	2.02 (1.5-2.4)	0.8 (0.6-1.1)	1.2 (0.9-1.5)	8 (6.6-11)	13 (8.5-22)
	CFA	**21**	7.0 (6.0-9)	47 (53-44)	60 (53-75)	15 (7-27)	1.7 (1.3-2.0) *	0.7 (0.5-0.9) *	1.0 (0.8-1.3) **	7 (4.6-8)	10 (6.3-16)

Table 2. A summary of the changes in electrophysiological properties of nociceptive DRG neurons 4 days after CFA-induced hind limb inflammation in the guinea pig and rat

CV range	Animal Group	N	CV m/s	Em - (mV)	AP Height (mV)	AP Overshoot (mV)	AP duration at base (ms)	Rise Time (ms)	Fall Time (ms)	AHP depth (mV)	AHP duration 80% (ms)
C-fiber	Rat Normal	34	—	—	—	—	—	—	—	—	—
	Rat CFA	43	—	—	—	—	—	—	—	—	—
	GP Normal	41	—	—	—	—	↓↓	↓	↓↓	—	↓
	GP CFA	23	↑	—	—	—				—	
Aδ-fiber	Rat Normal	58	—	—	—	—	—	—	—	—	—
	Rat CFA	24	—	—	—	—	—	—	—	—	—
	GP Normal	50	—	—	—	—	—	—	—	—	—
	GP CFA	19					↓↓	—	↓↓		↓

			—	—	—	—		↓		—	
Aα/β-fiber	Rat Normal Rat CFA	40 26	— —	— —	— —	— —	— —	— —	— —	— —	— —
	GP Normal GP CFA	51 21	— —	— —	— —	— —	— ↓	— ↓	— ↓↓	— —	— —

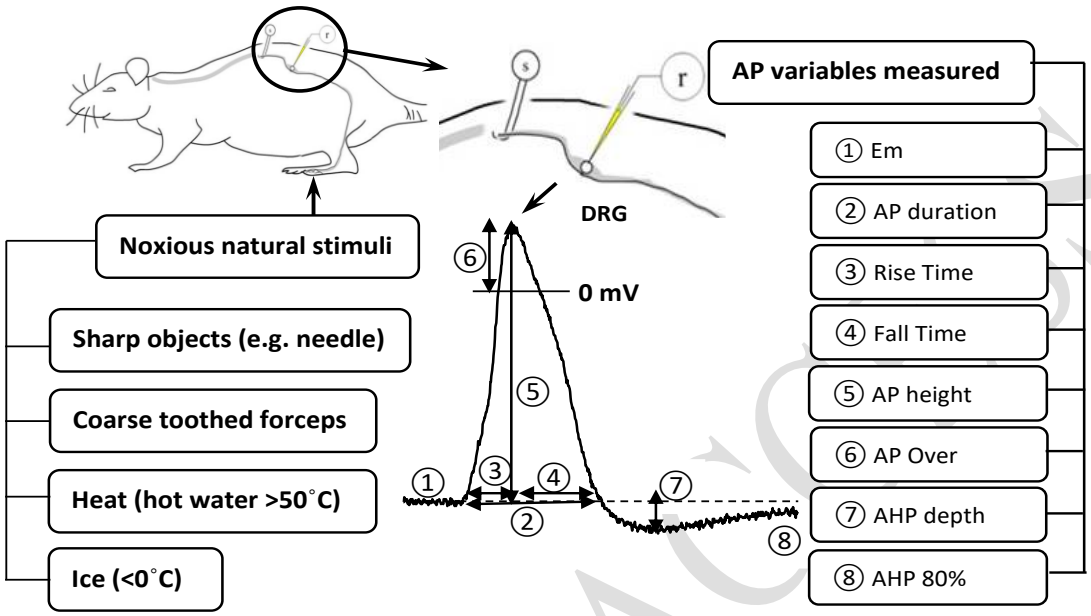

Figure 1. **A diagram showing the *in vivo* intracellular recording setup.** A glass microelectrode is inserted into a lumbar DRG neuron for intracellular recording (r) of somatic APs evoked antidromically by electrical stimulation of the dorsal root with a pair of bipolar platinum stimulating electrodes (s). The numbers on the intracellularly recorded somatic AP (middle) show the AP variables measured: (1) membrane potential (Em), (2) AP duration at base, (3) AP rise time, (4) AP fall time, (5) AP height/amplitude, (6) AP overshoot, (7) AHP depth and (8) AHP 80% (AHP duration to 80% recovery). The diagram also shows (left) the various noxious mechanical and thermal stimuli that were applied to the left hindlimb to classify neurons into different subtypes of nociceptors. AP = action potential; DRG = dorsal root ganglion.

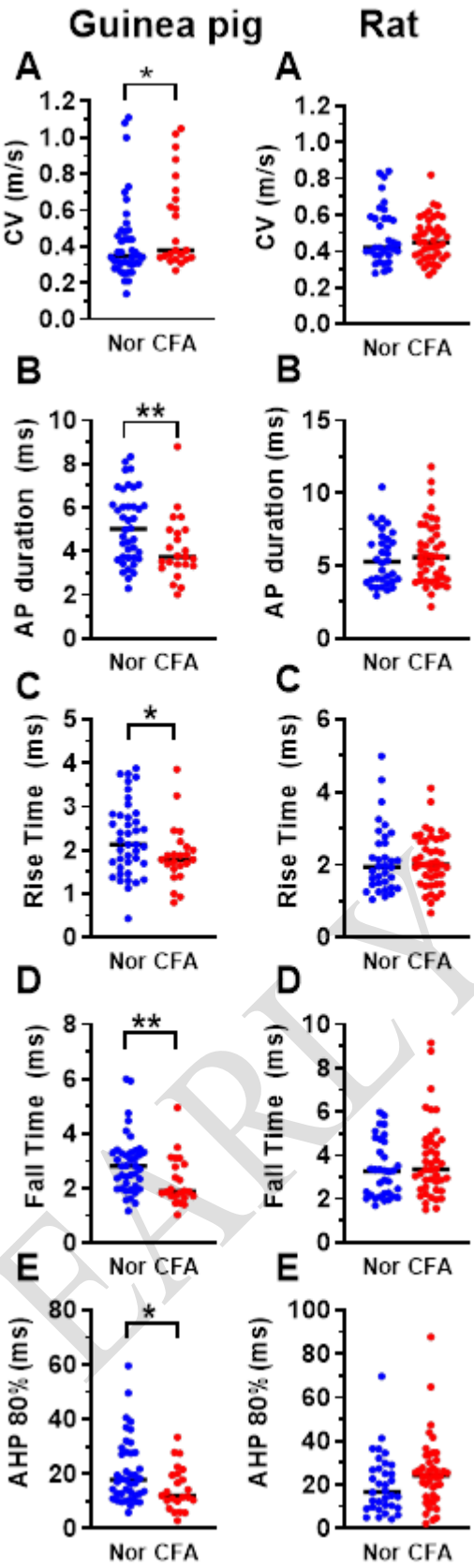

Figure 2. Impact of CFA on CV and AP variables in C-fiber nociceptive neurons in the guinea pig and rat. Scatterplots showing the effects of CFA treatment on CV (A) and AP variables that changed significantly in C-nociceptive neurons: AP duration at base (A), Rise time (C), Fall time (D) and AHP 80% (E). Each dot represents the value for one DRG neuron. Nor means untreated/normal animals, and CFA means CFA injection 4 days prior to the electrophysiological experiments. The median (horizontal line) is superimposed in each case, and the level of significance of any difference between normal animals (Nor) and CFA treated animals (CFA), is indicated by asterisks above the graphs (no asterisks indicate no significant differences). Note that the median values of the variables shown changed significantly in the guinea pig (left panel), but not in the rat (right panel). Comparisons between normal and CFA groups were made with the Mann–Whitney U test. The level of statistical significance is as follows: $*p < 0.05$; $**p<0.01$.

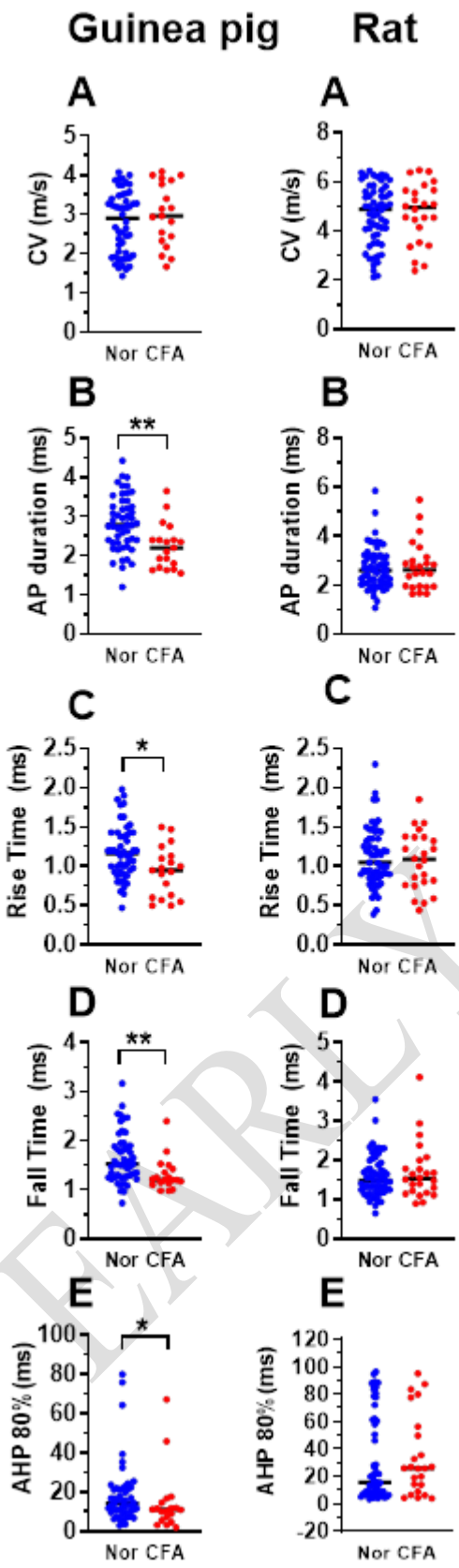

Figure 3. Impact of CFA on CV and AP variables in δ-fiber nociceptive neurons in the guinea pig and rat. Scatterplots showing the effects of CFA treatment on CV (A) and AP variables that changed significantly in δ-nociceptors namely AP duration at base (A), Rise time (C), Fall time (D) and AHP 80% (E). Like C-fiber nociceptors, the median values of these variables changed significantly in the guinea pig (left panel), but not in the rat (right panel). Each dot represents the value for one DRG neuron. Nor means untreated/normal animals, and CFA means CFA injection 4 days prior to the electrophysiological experiments. The median (horizontal line) is superimposed in each case, and the level of significance of any difference between normal animals (Nor) and CFA treated animals (CFA), is indicated by asterisks above the graphs (no asterisks indicate no significant differences). Note that the median values of the variables shown changed significantly in the guinea pig (left panel), but not in the rat (right panel). Comparisons between normal and CFA groups were made with the Mann–Whitney U test. The level of statistical significance is as follows: $*p < 0.05$; $**p<0.01$.

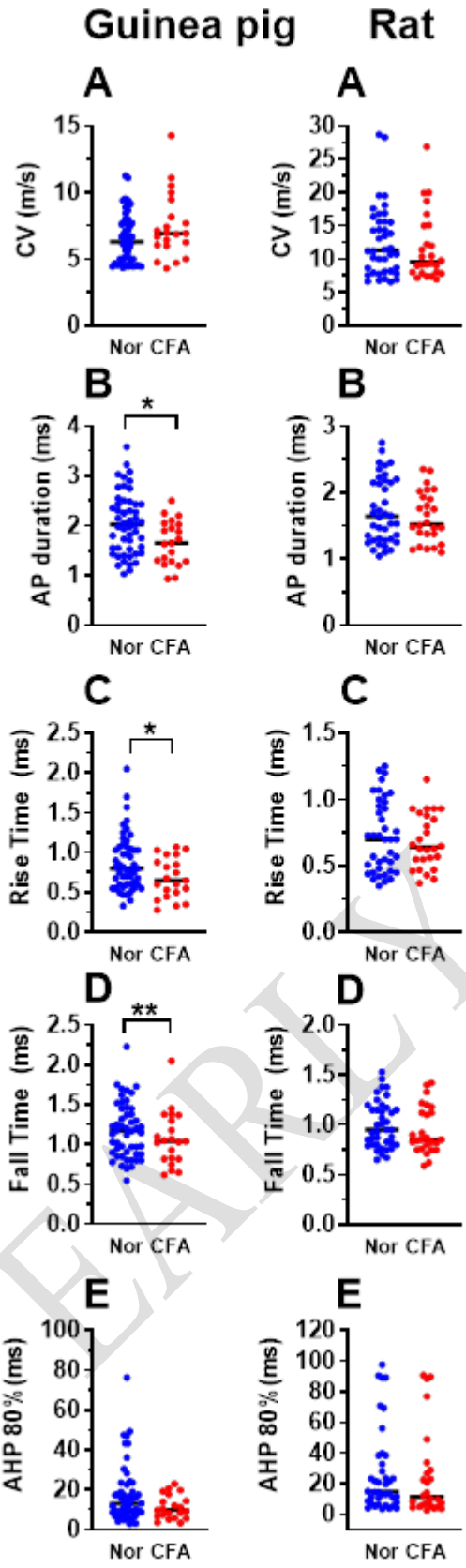

Figure 4. Impact of CFA on CV and AP variables in α/β-fiber nociceptive neurons in the guinea pig and rat. Scatterplots showing the effects of CFA treatment on CV (A) and AP

variables that changed significantly in α/β-fiber nociceptive DRG neurons which are: AP duration at base (A), Rise time (C) and Fall time (D. Like C-fiber nociceptors, the median values of these variables changed significantly in the guinea pig (left panel), but not in the rat (right panel). Each dot represents the value for one DRG neuron. Nor means untreated/normal animals, and CFA means CFA injection 4 days prior to the electrophysiological experiments. The median (horizontal line) is superimposed in each case, and the level of significance of any difference between normal animals (Nor) and CFA treated animals (CFA), is indicated by asterisks above the graphs (no asterisks indicate no significant differences). Note that the median values of the variables shown changed significantly in the guinea pig (left panel), but not in the rat (right panel). Comparisons between normal and CFA groups were made with the Mann–Whitney U test. The level of statistical significance is as follows: $*p < 0.05$; $**p<0.01$.