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Original article

# Dorsal horn disinhibition and movement-induced behaviour in a rat model of inflammatory arthritis

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## Abstract

**Objectives.** Alterations beyond joint inflammation such as changes in dorsal horn (DH) excitability contribute to pain in inflammatory arthritis (IA). More complete understanding of specific underlying mechanisms will be important to define novel targets for the treatment of IA pain. Pre-clinical models are useful, but relevant pain assays are vital for successful clinical translation. For this purpose, a method is presented to assess movement-induced pain-related behaviour changes that was subsequently used to investigate DH disinhibition in IA.

**Methods.** IA was induced by intra-articular injection of complete Freund's adjuvant (CFA) in male rats, and weight distribution was assessed before and after walking on a treadmill. To confirm increased activity in nociception-related pathways, fos expression was assessed in the superficial DH, including in nociceptive neurons, identified by neurokinin 1 (NK1) immunoreactivity, and interneurons. Inhibitory terminal density onto $NK1^+$ neurons was assessed and lastly, a cohort of animals was treated for 3 days with gabapentin.

**Results.** At 4 weeks post-CFA, walking reduced weight distribution to the affected joint and increased DH fos expression, including in $NK1^+$ neurons. Neuronal activity in inhibitory cells and inhibitory terminal density on $NK1^+$ neurons were decreased in CFA-treated animals compared with controls. Treatment with gabapentin led to recovered behaviour and DH neuronal activity pattern in CFA-treated animals.

**Conclusion.** We describe an assay to assess movement-induced pain-related behaviour changes in a rodent IA model. Furthermore, our results suggest that disinhibition may contribute to pain related to movement in IA.

**Key words:** arthritis, behaviour, spinal cord, inhibition, nervous system

BASIC SCIENCE

**Rheumatology key messages**

- We present a novel paradigm for clinically relevant pre-clinical behavioural assessment in an IA model.
- Inhibitory input onto pain-related neurons decreased and gabapentin attenuated behaviour in a joint pain model.
- Together these data support a dorsal horn disinhibition contribution to chronic joint pain.

## Introduction

Arthritis patients consistently complain of pain, but treatment can be difficult (reviewed in [1]) due largely to limited knowledge of pain mechanisms underlying chronic joint pain. Inflammatory arthritis (IA) patient outlook has vastly improved since the development of biologics, which often control inflammation and prevent joint damage [2]. Unfortunately, even with these disease features controlled, pain may persist [3]. This suggests, and certainly it is increasingly recognized, that mechanisms beyond the classical disease hallmarks of joint inflammation contribute to the pain of IA, such as neuroplastic changes in the CNS [4]. Recently we observed evidence of disinhibition in the dorsal horn (DH) [5], the first CNS site to receive pain-related (nociceptive) information from the periphery. In the DH, inhibitory and excitatory interneurons modulate sensory information before it is relayed by projections to higher centres. This work further investigates alterations that could modify the DH balance of excitation to inhibition and how this may relate to joint pain.

The understanding of joint pain mechanisms remains incomplete and, accordingly, the search for novel treatments has been problematic. Promising, specifically

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developed biologics that successfully reduce overt inflammatory and joint degradation processes, even those with a superior pain-relief compared with placebo, can leave many patients with ongoing pain [6]. Pre-clinical joint pain models offer a valid opportunity to investigate mechanistic pathways [7] but their usefulness has been questioned [8]. There is an agreement that improvement of how models are used [9], including relevant pain-detection assays, is vital for successful clinical translation [10]. In particular, emphasis has shifted from reflexive measurements, often from cutaneous stimulus application, towards non-reflexive measurements [11, 12]. This is particularly relevant to pre-clinical joint pain assessment as responses to those stimuli reconcile poorly with clinically reported joint pain qualities [13–15].

The first primary objective of this study was to model and assess movement-associated pain preclinically [14] by combining weight bearing analyses with a walking paradigm, in the complete Freund's adjuvant (CFA) IA rat model. The hypothesis was that, following walking, weight distribution to the affected paw would be reduced. This behavioural assessment was supplemented with DH fos expression analysis to confirm the activation of pain-related (nociceptive) pathways, including in neurons identified by expression of the substance P receptor neurokinin 1 (NK1), which are known to be important for nociceptive transmission [16, 17].

The second primary objective of this study was to investigate inhibitory apposition loss on NK1-expressing cells in the DH of CFA-treated animals. Secondary objectives were to assess movement-induced neuronal activity in inhibitory and excitatory DH interneurons in CFA-treated animals compared with control, and lastly to investigate the effect of gabapentin treatment on movement-induced behaviour and DH neuronal activity.

## Methods

### Ethical statement

All studies were approved by the McGill University Faculty of Medicine Animal Care Committee and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and checklist were consulted.

### Animals and induction of arthritis

Male Sprague–Dawley rats (purchased from Charles River, Wilmington, Massachusetts, USA), weighing 175 g at arrival, were housed two per cage with soft bedding on a 12-h light/dark cycle with food and water available *ad libitum*. Under 5% isoflurane in $O_2$ anaesthesia, the right tibia-talus joint was injected with 40 μl containing 40 μg of CFA [18] (Sigma-Aldrich, Saint-Louis, Missouri, USA) or vehicle (for shams). For details on group allocation and blinding see Supplementary Material, available at *Rheumatology* online. The total number of animals used in this study was 80, including those used for independent replication.

### Assessment of static weight-bearing and walking paradigm

Pain-related behaviour induced by movement was assessed by static weight distribution (SWD) before and after a period of treadmill walking. SWD through both hind paws was assessed with an incapacitance meter (Model 600 series 8; IITC Life Science, Woodland Hills, California, USA) before and after a 30-min treadmill (Exer 3/6; Columbus Instruments, Columbus, Ohio, USA) walking session at 5.5 m/min. More details on the behavioural paradigm can be found in Supplementary Material, available at *Rheumatology* online.

#### *Drug treatment*

Beginning at 25 days post-CFA (3 days before the experimental endpoint of 4 weeks), CFA-treated animals were injected i.p. once a day with 100 mg/kg of gabapentin (Spectrum Chemical Mfg. Corp., New Brunswick, New Jersey, USA) dissolved in sterile saline, or vehicle, for 3 days ($n = 10$). This dose was selected based on previous publications that found this to be effective at relieving neuropathic pain-related behaviour in rats without causing sedation [19].

### Tissue preparation

At 4 weeks post-CFA, animals were sacrificed 2 h after completing the walking period, as peak DH fos induction following noxious stimuli has been demonstrated at 2 h post-stimulus [20], with cohorts of sham and CFA-treated animals that did not walk as controls. Animals were anaesthetized and perfused with fixative, and spinal cords extracted and prepared for immunohistochemistry (IHC) (for detailed methods see Supplementary Material, available at *Rheumatology* online).

#### *Immunohistochemistry*

After washing and blocking, sections were incubated in primary antibodies against the following targets: fos to detect neuronal activity, paired box 2 (Pax2) to identify inhibitory interneurons, anti-vesicular γ-aminobutyric acid (GABA) transporter (VGAT) to identify inhibitory appositions, vesicular glutamate-2 (VGLUT2) and NK1 (the substance P receptor) for identification of nociceptive neurons. After washing and species-matched secondary antibody incubation, sections were incubated with a fluorescent Nissl stain to identify neuronal somata. Sections were washed, mounted on slides and coverslipped for later microscopy. For detailed IHC methods (including specific antibody details) see Supplementary Material, available at *Rheumatology* online.

#### *Microscopy*

Fos and Pax2 immunoreactivities were captured in transverse DH sections using a Zeiss AxioImager M2 Imaging microscope equipped with a digital camera, with Zeiss ZenPro software v.2.3 (Zeiss Canada Ltd, Toronto, Ontario, Canada). Initial analyses of fos signals were conducted on images captured with a ×10 objective, spanning the superficial DH medio-lateral extent.

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Later, fos and Pax2 analyses in the pharmacology experiment were performed on images captured with a ×20 objective from the medio-central superficial DH, where primary afferents innervating the ankle joint are known to terminate [21] and where alterations have previously been observed in this model [5]. Images of NK1-expressing ($NK1^+$) neurons in horizontal sections were acquired from medio-central lamina (L) I using Zeiss LSM750 and LSM800 confocal microscopes with a ×63 oil immersion objective. Z-series of 9 μm-thick optical sections with a 0.5 μm step were acquired for inhibitory and excitatory apposition density analysis. Consistent settings were used across all groups within each analysis.

### Image analysis

ImageJ was used to analyse fos and Pax2 expression, and from each animal three to six sections were analysed ($n = 8$ for the initial analysis and $n = 5$ for the pharmacology experiment). Laminar boundaries were overlaid and the fos signal first visualized alone to manually count positive cells. Then, the Pax2 channel was visualized to count double-labelled cells, expressed as a percentage. Separately, to count the total number of $Pax2^+$ neurons, the Analyze Particles tool was used.

Images were scanned with only the NK1 and Nissl signals, to first select cell bodies based on NK1 immunoreactivity and then to subsequently analyse nuclei for fos expression. A minimum of six $NK1^+$ cell bodies were analysed per animal. For apposition analysis onto $NK1^+$ cells, z-stacks were imported into Imaris (Bitplane AG, Zürich, Switzerland) image analysis software, and brightness and contrast were adjusted so that the $NK1^+$ membrane and apposition puncta could be clearly delineated. A 3D volume surface rendering of the NK1 signal was created to determine the volume of $NK1^+$ membrane. A mask of VGAT and VGLUT2 apposition puncta was created and counted using the Spots tool. To account for differences in the volume of $NK1^+$ membrane between images, the number of appositions per volume of $NK1^+$ membrane was calculated for each stack (two z-stacks per section, three sections per animal and $n = 3$–4).

### Experimental design and statistical analyses

Sample sizes were based on previous studies from our lab (including preliminary studies of the work present here), discussion with field experts, and standard practices in the preclinical pain field [22]. We conducted *post hoc* power analyses of data to confirm appropriateness. All data are presented as group mean (S.E.M.) calculated from individual animal means and were analysed with Windows GraphPad Prism Version 5 (GraphPad Software, La Jolla, CA, USA). Behaviour data and cell counts comparing fos signal pre- and post-walking in sham and CFA animals were analysed by repeated two-way analysis of variance (ANOVA), and the of IHC data with a one-way ANOVA. Bonferroni *post hoc* tests with an *a priori* level of significance of 95% were used.

## Results

### Effect of walking on weight distribution in CFA-treated and sham animals

To test the hypothesis that walking would lead to a decrease in weight distribution to the affected paw after intra-articular CFA injection, the weight placed on each hind paw was measured immediately before and after a period of walking on a treadmill. All animals were able to maintain pace for the 30 min. During this time the animals tended not to walk continuously but instead would walk faster than the speed of the treadmill, stop at the end to be carried back to the beginning and then resume walking back to the end (Supplementary Videos S1 and S2, available at *Rheumatology* online). Somewhat fascinatingly, occasionally animals would walk backwards at the edge of the beginning of the treadmill (Supplementary Video S3, available at *Rheumatology* online). These walking habits were observed in all the groups, at all time points examined.

Pre- and post-walking ipsilateral SWDs were equal for all groups at baseline and sham animals at all time points, with animals placing equal weight on each paw (Fig. 1A ). At weeks 1 and 2 post-CFA, pre-walking ipsilateral SWD was lower than at baseline, and lower than sham ipsilateral measurements ($P < 0.0001$; for a more complete statistical report see Supplementary Material, available at *Rheumatology* online). At these early time points, walking did not affect ipsilateral SWD and a pronounced limp during the walking period was noticeable (Supplementary Video S4, available at *Rheumatology* online). By 3 weeks post-CFA the limp was less marked, and no longer present by 4 weeks post-CFA. Also beginning at 3 weeks post-CFA, there was a recovery of pre-walking measurements towards equal distribution across both hind paws that was complete by 4 weeks (Fig. 1A). Despite the lack of altered SWD post-walking at early post-CFA time points, by 4 weeks post-CFA post-walking ipsilateral SWD was strongly decreased compared with pre-walking in CFA-treated animals ($P = 0.002$). Groups including ours have described that arthritis-related changes emerge in this model only at later time points [5], and for these reasons 4 weeks post-CFA was selected for subsequent histology and pharmacology studies.

### Neuronal activity in the superficial dorsal horn after walking is altered in CFA-treated animals

Activity of nociception-associated neuronal pathways following walking was validated by DH fos expression [23] (Fig. 1B and C). As expected, there were low levels of, if any, fos immunoreactivity in the DH of sham animals that had remained under normal conditions. Numbers of $fos^+$ cells between groups that did not walk were not statistically different. There was an increase in

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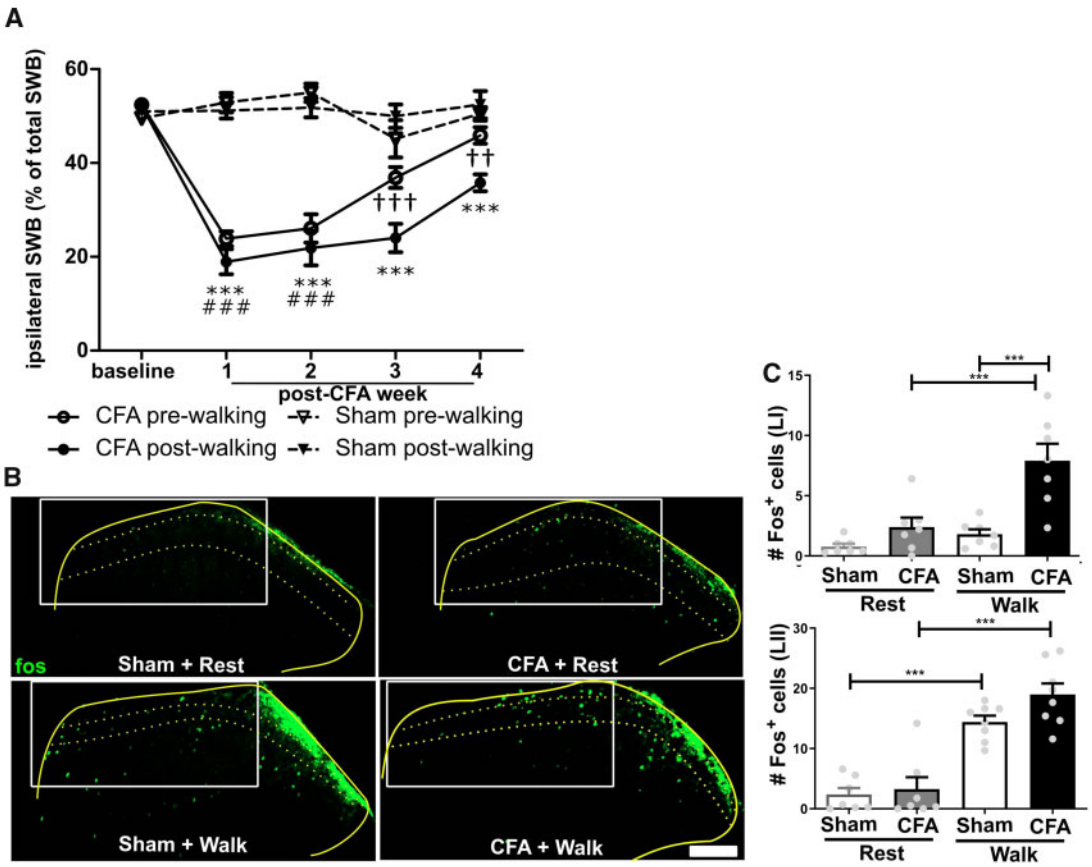
FIG. 1 Walking induces behaviour and DH fos expression indicative of nociception in CFA-treated animals




(**A**) Ipsilateral SWD before and after 30 min of walking on a treadmill at 5.5 m/min. Points represent mean (S.E.M.) of SWD. Two-way ANOVA with Bonferroni-corrected *t*-test: ###$P < 0.001$ pre-walking CFA *vs* sham, ***$P < 0.001$ post-walking CFA *vs* sham, †††$P < 0.001$, †† $P < 0.01$ CFA pre-walking *vs* post-walking; $n = 10$. (**B**, **C**) DH fos expression (green). (**B**) DH outline is shown in solid yellow and the dotted lines show LI–LII and LII–LII boundaries. (**C**) Counts of fos$^+$ cells in LI and LII of ipsilateral DH. Bars represent mean (S.E.M.) of ipsilateral fos$^+$ counts. Two-way ANOVA with Bonferroni-corrected *t*-test: ***$P < 0.001$; $n = 6$–8. The box shows the region of the DH that was examined in subsequent IHC analysis. Scale bar: 100 μm. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; DH: dorsal horn; L: lamina; SWD: static weight distribution.

the number of fos$^+$ neurons in LI of CFA-treated animals post-walking compared with sham animals and CFA-treated animals that had not walked ($P = 0.0001$), and we observed that this was concentrated towards the medio-central zone of LI. To further support this suggestion that walking for 30 min is a noxious stimulus for CFA-treated animals, fos expression in NK1$^+$ cells was analysed as these are crucial for nociception transduction. The proportion of double labelled NK1$^+$ and fos$^+$ cells was higher in CFA-treated compared with sham DH post-walking (Fig. 2; $P = 0.04$). Also observable in the DH of CFA-treated animals was an increase in overall NK1 immunoreactivity, which we did not quantify. This increase was clearly apparent in all sections examined.

Walking induced fos expression in LII in sham and CFA-treated animals (Figs 1–3). Interestingly, fewer less fos$^+$ cells were inhibitory in CFA animals compared with sham animals (Fig. 3; $P = 0.0033$), but there was no difference in the overall number of Pax2-positive neurons in the DH of CFA-treated animals compared with control.

## Gabapentin ameliorates the weight-bearing deficit induced by walking

The hypothesis was tested that gabapentin, a first-line drug for the treatment of neuropathic pain [24], could attenuate the movement-associated behaviour deficit. A cohort of CFA-treated animals were administered daily gabapentin for 3 days, starting at 25 days post-CFA, until the 4-week experimental endpoint, at which time, pre- and post-walking SWD of CFA-vehicle, CFA-gabapentin and sham animals were assessed and animals were sacrificed 2 h later.

As in the previous experiment, sham animal weight distribution was equal across each hind-paw at pre- and

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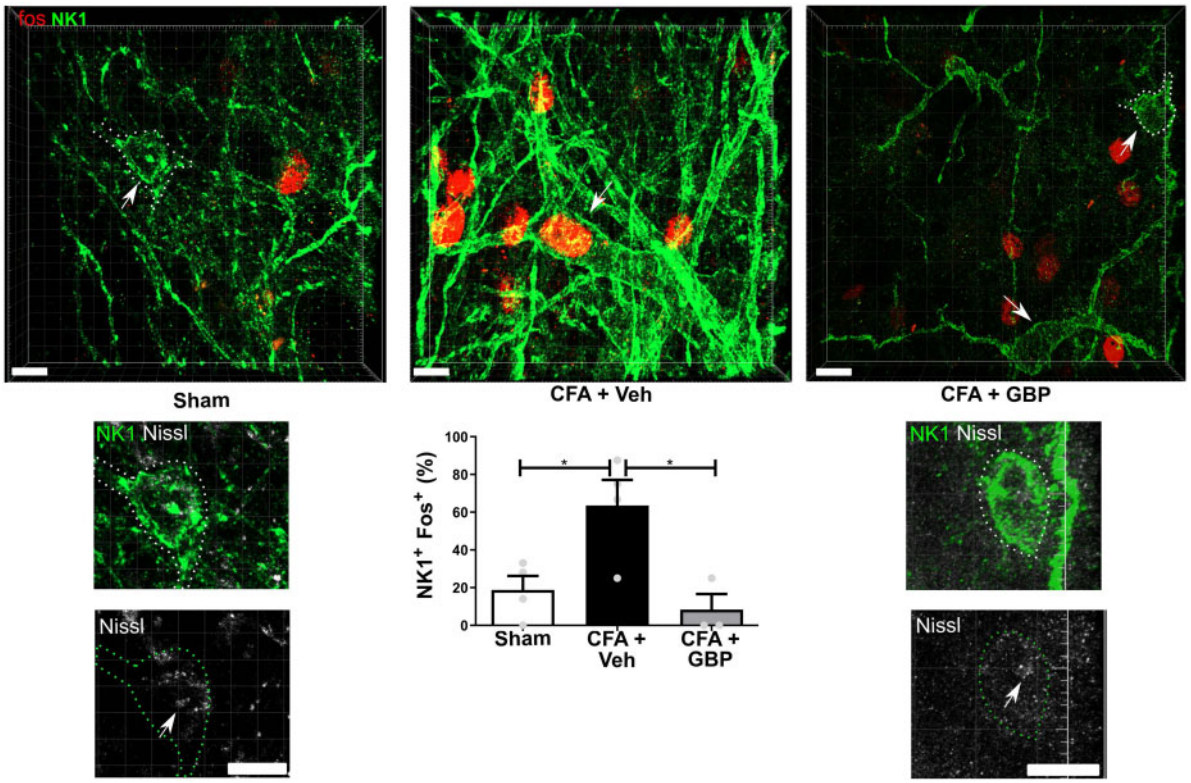
Fig. 2 Walking results in dorsal horn fos expression in nociceptive neurons in CFA-treated animals




Fos expression (red) in NK1 (green)-expressing cell nuclei. Arrows indicate NK1$^+$ cells, with dotted lines indicating fos-negative cells that are enlarged below where Nissl staining is shown (white). In the lowest panels, the arrow points to the nucleolus. This is quantified in the graph as percentage of examined NK1 expressing (NK1$^+$) neurons that were also fos$^+$. Bars represent mean (S.E.M.) of ipsilateral fos$^+$ counts. One-way ANOVA with Bonferroni-corrected *t* test: $^*P < 0.05$; $n = 3$–5. Scale bar: 10 μm. Notice the increased NK1 immunoreactivity in CFA-treated animals and its reduction after gabapentin treatment. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; GBP: gabapentin; NK1: neurokinin 1.

post-walking assessments (Fig. 4). Also, in agreement with our previous data from 4 weeks post-CFA, pre-walking assessment showed equal weight distribution across the hind paws of CFA-treated animals, but ipsilateral paw post-walking SWD was reduced ($P = 0.0014$). Remarkably, gabapentin treatment prevented the reduced ipsilateral post-walking SWD (Fig. 4; $P = 0.0052$).

## Gabapentin rescues dorsal horn neuronal activity profile

As expected, LI fos expression in CFA animals was reduced by gabapentin to sham levels (Fig. 5A and B; $P = 0.0150$). Also in agreement with the hypothesis that gabapentin would reduce movement-induced nociception in CFA-animals, the proportion of cells double labelled for NK1 and fos was decreased in the gabapentin-treated group to sham levels (Fig. 2B; $P = 0.0251$).

In LII, there was no difference between groups in the number of fos$^+$ cells (Fig. 5A and C; $F_{(2,10)} = 0.1954$, $P < 0.8256$). Similar to the previous experiment, the proportion of fos$^+$ cells that were inhibitory in CFA-vehicle animals was decreased compared with sham and this was recovered in CFA-gabapentin animals (Fig. 5D and E; $P = 0.0011$).

## Reduction in inhibitory input to NK1-expressing cells

Using 3D reconstructions of confocal stacks, VGAT-immunoreactive boutons per volume of NK1 immunoreactivity were assessed to investigate inhibitory apposition density onto NK1$^+$ cells. CFA treatment was associated with reduced NK1$^+$ inhibitory terminal density (Fig. 6A; $P = 0.001$), which was unaffected by gabapentin treatment ($P = 0.5187$).

There were no significant changes detected in excitatory apposition density onto NK1-positive cells (Fig. 6B; $P = 0.1188$). However, there was a trend towards an



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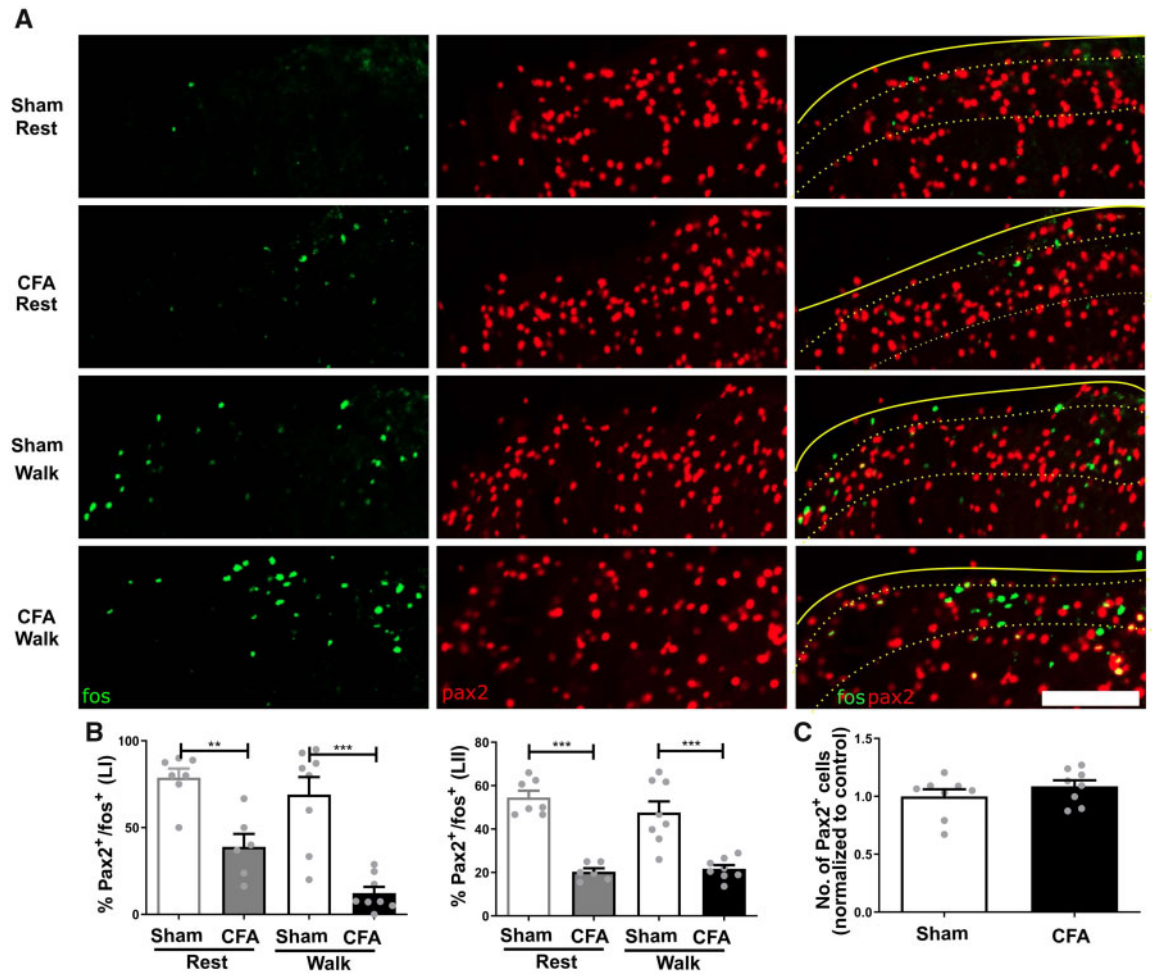
FIG. 3 Fos expression profiles are altered in the dorsal horn of CFA-treated animals




(**A**) Fos expression (fos$^+$) in dorsal horn neuron cell bodies can be seen in green and Pax2 expression (Pax2$^+$) in red. The outline of the dorsal horn is shown in solid yellow and the dotted lines show the LI–LII and LII–LIII boundaries. (**B**) The percentage of fos$^+$ cell bodies that were also Pax2$^+$ in LI and LII of ipsilateral dorsal horns. Bars represent mean (S.E.M.). One-way ANOVA with Bonferroni-corrected *t*-test: $^{***}P < 0.001$, $^{**}P < 0.01$; $n = 6$–8. Scale bar: 100 μm. (**C**) Total Pax2 cell counts. Bars represent mean (S.E.M.), normalized to sham. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; L: lamina.

increase in CFA-vehicle animals and prevention of this with gabapentin treatment.

## Discussion

This study had two main objectives: to develop a clinically relevant method to quantify joint pain, and to investigate DH disinhibition in CFA-treated animals. We demonstrate that in CFA-treated animals walking provoked a weight-bearing deficit on the affected paw and a superficial DH pattern of fos expression typical of a nociceptive stimulus, including expression in nociceptive cells. Decreased inhibitory apposition density onto NK1$^+$ cells and altered fos expression in inhibitory neurons were observed in CFA-treated animals compared with sham. Lastly, at 4 weeks post-CFA in gabapentin-treated animals, both the behaviour and fos pattern resembled that observed in shams.

At early time points there was a robust reduction in ipsilateral pre-walking CFA SWD compared with control, which was unchanged by walking. At the same time points animals were observed to walk with an altered gait, spending less time on the affected paw while walking. The observed recovery in pre-walking SWD is not likely due to weekly walking as we observed the same effect in a cohort that only walked during habituation and at the end point. The resolution of pre-walking SWD parallels global paw inflammation, paw guarding and gait. Therefore, it is difficult to tell if the equal pre- and post-walking SWD at these early time points is due to a maximal effect in pre-walking assessments, or because

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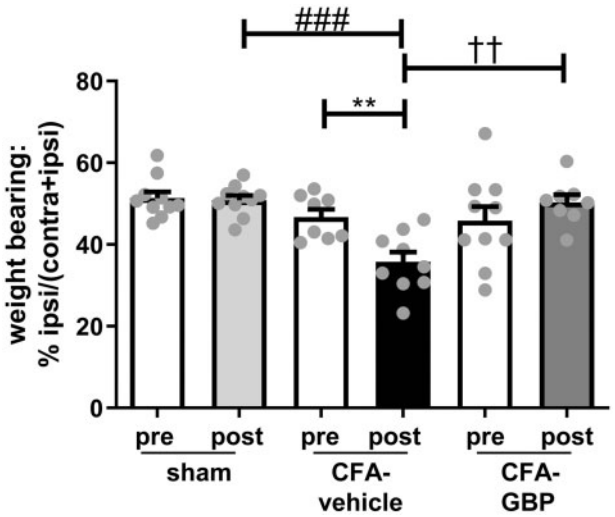
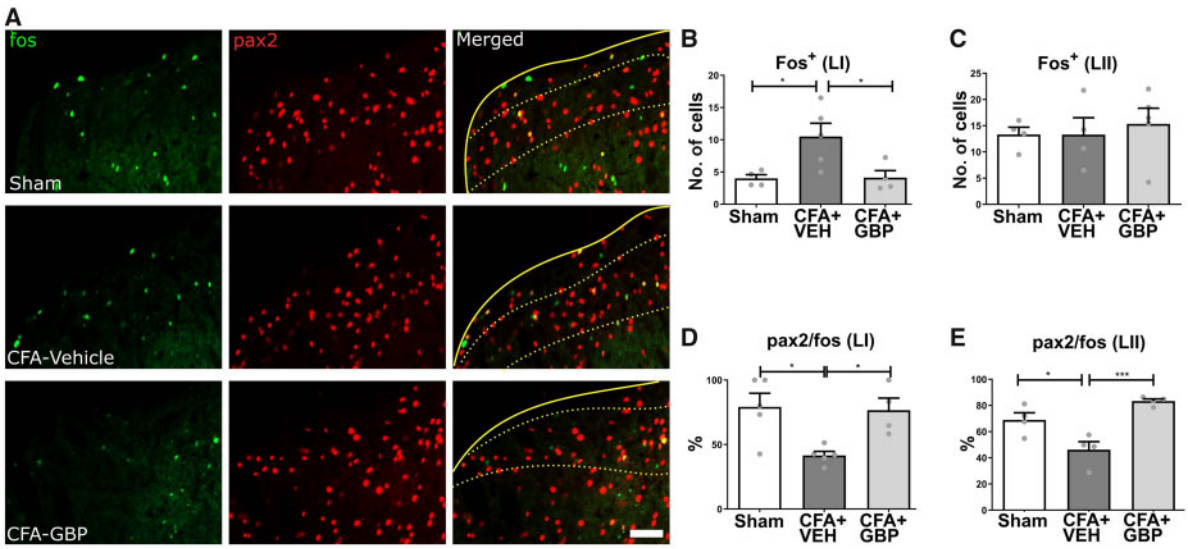
Fig. 4 Movement-induced behaviour in CFA-treated rats is attenuated by gabapentin




Weight-bearing on the ipsilateral paw was assessed pre- and post-walking on a treadmill at 5.5 m/min for 30 min. Bars represent mean (S.E.M.) of ipsilateral SWD (as a percentage of total SWD). Two-way ANOVA with Bonferroni-corrected *t*-test: ###$P < 0.0001$ post-walking CFA *vs* sham, **$P < 0.01$, CFA-vehicle post- *vs* pre-walking, ††$P < 0.01$, post-walking CFA-vehicle *vs* CFA-gabapentin; $n = 10$. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; GBP: gabapentin.

Fig. 5 Altered dorsal horn fos expression profiles in CFA-treated animals is normalized by gabapentin treatment




(**A**) Fos expression (fos$^+$; green) and Pax2 expression (Pax2$^+$; red) in dorsal horn neuron cell bodies. The outline of the dorsal horn is shown in solid yellow and the dotted lines show the LI–LII and LII–LIII boundaries. (**B**) The number of fos$^+$ cell bodies in LI. (**C**) LII. (**D**, **E**) The percentage of fos$^+$ cell bodies that were double-labelled for Pax2. Bars represent mean (S.E.M.). One-way ANOVA with Bonferroni-corrected *t*-test: ***$P < 0.001$, *$P < 0.05$; $n = 4$–5. Scale bar: 50 μm. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; GBP: gabapentin; L: lamina.

the altered gait leads to avoidance of further discomfort. Most importantly, at the end point of 4 weeks post-CFA, post-walking SWD was lower than pre-walking. At this time the pattern of fos immunoreactivity in the superficial DH following walking in CFA-treated animals was consistent with noxious stimuli (for a review of fos and nociception in the DH see [24]) whereas in sham animals this agreed with the pattern previously observed



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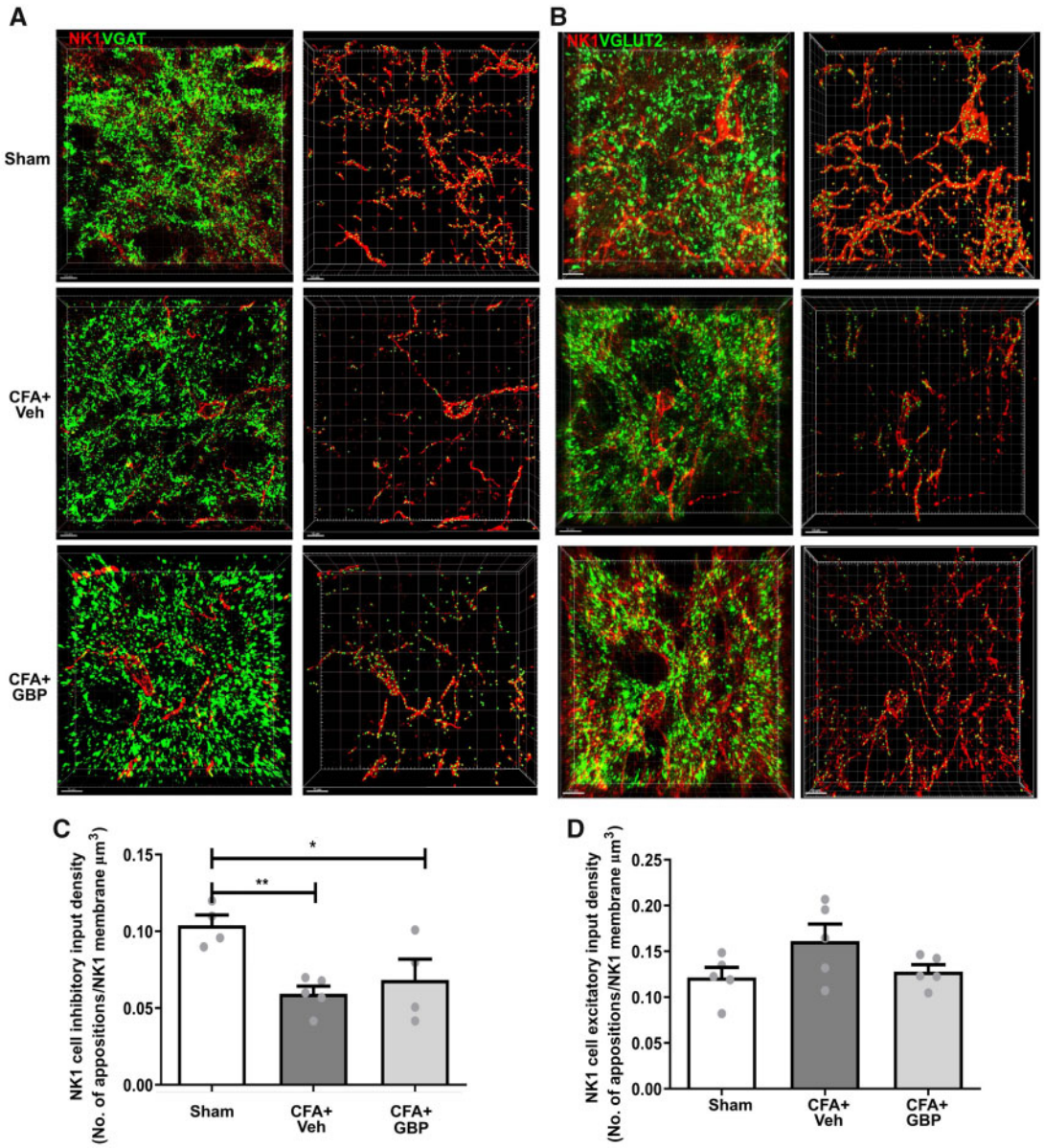
FIG. 6 Inhibitory terminal density on NK1-expressing cells is decreased in the dorsal horn of CFA-treated animals




(**A, B**, left) Density (green) on NK1 (red) neurons of appositions from inhibitory (**A**) and excitatory (**B**) terminals (boutons). Right: surface renderings of the NK1$^+$ membrane; green spots represent boutons that lie on the NK1$^+$ membrane. (**C**, **D**) Bars representing mean (S.E.M.) of bouton density on NK1$^+$ cells from inhibitory (**C**) and excitatory (**D**) terminals (boutons). One-way ANOVA with Bonferroni-corrected *t*-test: $^{**}P < 0.01$, $^{*}P < 0.05$; $n = 4$–5. ANOVA: analysis of variance; CFA: complete Freund's adjuvant; GBP: gabapentin; NK1: neurokinin 1.

following walking [25, 26]. Further, the distribution of fos expression observed here in the medial two-thirds of the DH corresponds to where primary afferents that innervate the ankle joint terminate [21]. We interpret this to suggest that our walking paradigm leads to activation of nociceptive signals originating from the inflamed ankle joint. As NK1 expression is indicative of nociceptive cells [16, 17], the final piece of evidence that our walking paradigm leads to activity in nociceptive pathways in CFA-treated animals is the increased percentage of NK1$^+$ cells that expressed fos. Critically, rheumatology patients often complain of pain induced by activity [13] that severely limits quality of life and patient functioning, another primary clinical assessment strongly associated with pain [27, 28]. This underlies the clinical relevance of our paradigm, which will likely be useful in other models of arthritis as both aspects (the treadmill and incapacitance meter) have been successfully used individually

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[29, 30]. This is important as other functional assessments related to pain [31] have not been validated across models or species. Thus, we consider our first objective to have been achieved with this novel, clinically relevant way to assess pain-related behaviour originating from the ankle joint following movement.

The DH is a major site for somatosensory information integration where interneurons prevail, essential for appropriately modulating incoming sensory information [32]. Inhibitory interneurons control information flow from the DH mostly post-synaptically, via input onto dendrites and somata of spinal cord neurons [33]. Melzack and Wall's gate control theory [34, 35] linked reduced DH inhibition (disinhibition) to pain, and subsequent studies confirmed an important role in neuropathic pain [36–38]. Multiple contributing mechanisms are possible such as altered GABAergic tone [37], inhibitory synapse loss [36] or changes in GABA receptors. In neuropathic pain altered chloride extrusion from projection neurons has been implicated [39] and there has been controversy over whether there is significant inhibitory cell death [37, 40]. While addressing the second objective to investigate DH disinhibitions in CFA-treated animals, decreased inhibitory apposition density onto NK1-expressing cells was found with no apparent loss of inhibitory cell bodies. This builds on our previous finding that suggested a loss of DH inhibitory terminals in models of neuropathic pain [36] and arthritis [5], supporting a role for inhibitory synapse loss and disinhibition in arthritis. When double-labelled inhibitory neurons were analysed as a proportion of total $fos^+$ cells, this was found to be decreased in CFA-treated animals compared with sham animals. Due to circuitry complexity within the DH and with higher centres, interpretation of this is challenging. Noxious stimulation induces reciprocal supraspinal and DH fos expression (reviewed in [24]). Indeed, DH circuitry, including ascending, local and descending pathways, and how this changes in chronic pain remain mysterious [41]. Where central pain pathway aberrations driving this altered ratio lie cannot be determined with this assessment, particularly as it is known that chronic pain conditions including arthritis lead to widespread CNS alterations [42]. In this study anatomical markers of neuronal activity and inhibitory and excitatory input were used. Therefore, further investigations, including electrophysiological studies and anatomical analyses of synapses, will be necessary to fully understand how excitatory and inhibitory mechanisms are altered in arthritis.

The last objective was to demonstrate a beneficial effect of gabapentin on movement-induced behaviour in the CFA model of IA. Gabapentin binds to the $\alpha_2\delta$ subunits of voltage-gated calcium channels [43] and the antinociceptive effects in neuropathic pain are attributed primarily to binding to upregulated $\alpha_2\delta_1$ on primary afferents. However, many mechanisms of this drug have been investigated, including those originating in the peripheral and central nervous centres, both spinally and supraspinally [44]. Adding the uncertainty of gabapentin's mechanism to the complexities of DH circuitry creates difficulty drawing a specific mechanistic conclusion of the positive gabapentin effects in this study. Nonetheless, the attenuated SWD deficit and DH fos profiles recovered by gabapentin in CFA-treated animals are important and strengthen the concept that DH disinhibition may occur and contribute to movement-related pain in arthritis. The trend towards reduced excitatory apposition density with gabapentin is intriguing as gabapentin has been shown to block abnormal excitatory synaptogenesis in the spinal cord via binding to $\alpha_2\delta_1$ [45], but this has yet to be shown within the context of pain. This was not a primary goal of this study and therefore it is possible that a longer treatment time with different analyses may reveal a novel mechanism for the antinociceptive effect of gabapentin. With only 3 days of gabapentin treatment, an effect of inhibitory terminal loss was unexpected; however, prolonged earlier treatment would be interesting. Of course, only male rats were included and a replicable female effect is important.

These findings are in keeping with other functional studies that show changes in excitatory to inhibitory tone in the DH in arthritis [46, 47] and add to the suggested mechanistic overlap between pain in arthritis and neuropathic pain [42]. This is important because IA pain is classically considered to originate predominantly in the periphery. Unquestionably, joint alterations in the inflammatory milieu will induce activation and sensitization of peripheral nociceptors. Yet that pain often persists after inflammatory resolution and in the absence of structural damage implies that maladaptive nervous system alterations are relevant to IA pain. There is an increasing recognition that there may be common mechanisms underlying many chronic pain conditions, particularly in cases that are refractory to treatments that normally demonstrate widespread efficacy [46, 48]. The data presented in this paper suggest that the consideration of IA subtypes as one of a group of chronic pain diseases that involve nervous system alterations could lead to improved patient outcomes.

The weight distribution and walking paradigm presented here represents a novel method for the assessment of movement-induced behaviour in joint pain models. The behaviour paradigm described here can be used in preclinical arthritis modelling to elucidate mechanisms underlying clinically relevant pain-related behaviour. Indeed, optimal treatment for pain in arthritis will most likely only be achievable with a thorough understanding of the pathological processes, alterations and damage both within and beyond the joint. Together, the DH neuronal changes and gabapentin effects shown in this study add to the growing body of evidence that many chronic pain conditions including arthritis and neuropathic pain involve common pathological mechanisms.

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## Supplementary data

Supplementary data are available at *Rheumatology* online.

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