



Grip strength in mice with joint inflammation: A rheumatology function test sensitive to pain and analgesia



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ABSTRACT

Grip strength deficit is a measure of pain-induced functional disability in rheumatic disease. We tested whether this parameter and tactile allodynia, the standard pain measure in preclinical studies, show parallels in their response to analgesics and basic mechanisms. Mice with periarticular injections of complete Freund's adjuvant (CFA) in the ankles showed periarticular immune infiltration and synovial membrane alterations, together with pronounced grip strength deficits and tactile allodynia measured with von Frey hairs. However, inflammation-induced tactile allodynia lasted longer than grip strength alterations, and therefore did not drive the functional deficits. Oral administration of the opioid drugs oxycodone (1–8 mg/kg) and tramadol (10–80 mg/kg) induced a better recovery of grip strength than acetaminophen (40–320 mg/kg) or the nonsteroidal antiinflammatory drugs ibuprofen (10–80 mg/kg) or celecoxib (40–160 mg/kg); these results are consistent with their analgesic efficacy in humans. Functional impairment was generally a more sensitive indicator of drug-induced analgesia than tactile allodynia, as drug doses that attenuated grip strength deficits showed little or no effect on von Frey thresholds. Finally, ruthenium red (a nonselective TRP antagonist) or the *in vivo* ablation of TRPV1-expressing neurons with resiniferatoxin abolished tactile allodynia without altering grip strength deficits, indicating that the neurobiology of tactile allodynia and grip strength deficits differ. In conclusion, grip strength deficits are due to a distinct type of pain that reflects an important aspect of the human pain experience, and therefore merits further exploration in preclinical studies to improve the translation of new analgesics from bench to bedside.

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Abbreviations: ANOVA, analysis of variance; CFA, complete Freund's adjuvant; COX-2, cyclooxygenase-2; DRG, dorsal root ganglion; HPMC, hydroxypropyl methylcellulose; NSAID, nonsteroidal antiinflammatory drug; p.o., orally; QST, quantitative sensory testing; RR, ruthenium red; RTX, resiniferatoxin; s.c., subcutaneous; SC, spinal cord; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1.

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1. Introduction

Pain is an important global health problem, and there is a need for new analgesics (Goldberg and McGee, 2011). However, despite major advances in our understanding of pain mechanisms in recent decades, there has been little translation of new analgesics from bench to bedside (Barrett, 2015; Kissin, 2010; Mao, 2009).

The predictive validity of animal models of pain has been intensely debated, and one possible reason for the limited

translation of pain research is the differences in outcome measures used to evaluate pain and analgesia in experimental animals and human patients (Cobos and Portillo-Salido, 2013; Mogil and Crager, 2004; Negus et al., 2006; Negus, 2013; Percie and Rice, 2014). Ideally, for the purposes of translation, animal testing should mimic as closely as possible routine clinical practice and clinical trials. Standard outcome measures used in preclinical chronic pain research have been adapted from quantitative sensory testing (QST) designed for the evaluation of patients with chronic pain, and von Frey filaments are one of the most widely used QST instruments to determine the mechanical pain threshold in preclinical research. In human patients, QST procedures are used to detect sensory alterations during neuropathic pain (e.g. Bennett, 2001; Bouhassira et al., 2005; Moharic et al., 2012). However, the use of QST in patients with rheumatic diseases is rare. To our knowledge only three published studies used von Frey filaments in human patients with rheumatoid arthritis (Hendiani et al., 2003; Morris et al., 1997; van Laarhoven et al., 2013), one of the most worrisome painful conditions that occurs with joint inflammation (Lee, 2013; Scott et al., 2000). This low number of clinical reports with von Frey testing is in marked contrast to the hundreds of preclinical studies that have used this technique in animal models of joint pain.

Pain is a complex phenomenon. Part of the core of the human pain phenotype includes alterations in physical functioning, which negatively impacts several aspects of daily life in patients with painful diseases (Romera et al., 2011; Turner et al., 2005). Because of the important relationship between pain and physical functioning, one set of consensus-based recommendations advocates measuring physical function as one of the main outcomes in clinical trials of treatments for pain (Dworkin et al., 2008). In this connection, grip strength has been widely and routinely evaluated for decades in rheumatology as a functional measure in patients with joint inflammation (e.g. Bijlsma et al., 1987; Lee, 2013; Pincus and Callahan, 1992), and remarkably, it is known to correlate to pain (Callahan et al., 1987; Fraser et al., 1999; Overend et al., 1999). Despite the widespread use of grip strength in rheumatology, this outcome is poorly characterized as a pain measure in experimental animals. However, as noted above, preclinical studies of tactile allodynia are abundant. It is known that transient receptor potential (TRP) channels or TRP-expressing nociceptors participate in inflammatory cutaneous hypersensitivity (Szallasi et al., 2007), but much less is known about the neurobiological mechanisms leading to pain-induced functional disability.

In light of these antecedents, we aimed to compare the sensitivity of grip strength in mice with joint inflammation vs. inflammatory tactile allodynia to the effects of several analgesic drugs of different pharmacological classes, and tested whether the appearance of grip strength deficits and tactile allodynia arose from the same mechanisms.

2. Material and methods

2.1. Experimental animals

Experiments were done in 680 female CD1 mice (Charles River, Barcelona, Spain) weighing 28–30 g at the beginning of the study. We chose female animals because it has been reported that women may be at greater risk for pain-related disability than men (e.g. Unruh, 1996; Stubbs et al., 2010), but no previous studies have evaluated grip strength as a measure of pain-induced functional disability in female animals. Animals were tested randomly throughout the estrous cycle. They were housed in colony cages with free access to food and water prior to the experiments, and were kept in temperature- and light-controlled rooms (22 ± 2 °C, and light–dark cycle of 12 h). The experiments were done during

the light phase (from 9:00 a.m. to 3:00 p.m.). All experimental protocols were carried out in accordance with international standards (European Communities Council directive 2010/63), and were approved by the Research Ethics Committee of the University of Granada. To decrease the number of animals in this study, we used the same mice for behavioral studies, histological analysis and immunostaining, when possible.

2.2. CFA-induced periarticular inflammation

Mice were injected periarticularly with complete Freund's adjuvant (CFA) (Sigma-Aldrich, Madrid, Spain) or sterile physiological saline (0.9% NaCl) as a control around the tibiotarsal joint. CFA (or saline) was administered subcutaneously in two separate injections to the inner and outer side of the joint in a volume of 10 or 15 μ L/injection (20 or 30 μ L/paw), to obtain homogeneous inflammation (Chen et al., 2009; Lolignier et al., 2011). We used a 1710 TLL Hamilton microsyringe (Teknokroma, Barcelona, Spain) with a 30½-gauge needle under isoflurane anesthesia (IsoVet®, B. Braun, Barcelona, Spain). CFA-treated mice had prominent inflammation that appeared to be restricted to the administration site and nearby areas (heel), whereas the paw pad did not appear to be affected. This allowed us to test the mechanical threshold in these two distinct areas. See “Results” for details. Because weight loss or delayed weight gain are considered signs of ongoing distress (Blackburn-Munro, 2004), body weight was monitored daily to ensure that our protocol did not induce excessive harm to the animals. Inflammatory edema was monitored by measuring ankle thickness with an electronic caliper (e.g. Croci and Zarini, 2007).

2.3. Drugs and drug administration

We used the following prototypic analgesics: the nonsteroidal antiinflammatory drug (NSAID) ibuprofen sodium salt (10–80 mg/kg), the cyclooxygenase-2 (COX-2) inhibitor celecoxib (40–160 mg/kg), and acetaminophen (40–320 mg/kg) (all from Sigma-Aldrich), and the opioids tramadol (10–80 mg/kg) and oxycodone hydrochloride (1–8 mg/kg) (supplied by Laboratorios Esteve, Barcelona, Spain). We also tested the effects of the antispastic baclofen (5–20 mg/kg) (Sigma-Aldrich). All drugs were dissolved in 0.5% hydroxypropyl methylcellulose (HPMC) with the exception of celecoxib and acetaminophen, which were suspended in HPMC supplemented with 1% Tween 80 (both from Sigma-Aldrich). These drugs or their solvents were administered orally (p.o.) in a volume of 10 mL/kg.

In addition, we also tested the effects of ruthenium red (1–2 mg/kg) (Sigma-Aldrich), a nonselective TRP antagonist (St Pierre et al., 2009). Ruthenium red was dissolved in saline and administered subcutaneously (s.c.) into the interscapular zone in a volume of 5 mL/kg. The control group received an equal volume of saline.

In all cases, behavioral evaluations after drug administration were recorded by an observer blinded to the treatment.

2.4. In vivo ablation of TRP vanilloid 1 (TRPV1)-expressing nociceptive neurons

We used resiniferatoxin (RTX) to selectively ablate TRPV1-expressing neurons. The drug (Tocris Cookson Ltd, Bristol, UK) was dissolved in 10% Tween 80 and 10% ethanol in normal saline. Animals received a single dose of RTX (50 μ g/kg) via intraperitoneal injection, which has been previously reported to ablate all peripheral TRPV1+ neurons (Hsieh et al., 2012). The control group received an equal volume of vehicle. All procedures were done under isoflurane anesthesia to minimize distress, 5 days before behavioral testing or sample collection.

2.5. Measurement of grip strength

Grip strength was measured with a computerized grip strength meter (Model 47200, Ugo-Basile, Varese, Italy). The apparatus consisted of a T-shaped metal bar connected to a force transducer. To measure grip strength in the hindpaws of the mice, the experimenter held the mouse gently by the base of the tail, allowing the animal to grasp the metal bar with its hindpaws. To prevent mice from gripping the metal bar with their forepaws during the recording, the animals were first allowed to grasp a wire mesh cylinder with their forepaws. As soon as the mice grasped the transducer metal bar with their hindpaws, the experimenter pulled the animals backwards by the tail until grip was lost (see Supplemental Video, which demonstrates the procedure used to measure hindlimb grip strength). The peak force of each measurement was automatically recorded in grams (g) by the device. Hindlimb grip strength in each mouse was measured in triplicate. Basal grip strength values were recorded for each animal as the average of two determinations on different days before the administration of CFA or saline. This value was considered as 100% of grip strength and used as a reference for subsequent determinations.

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.neuropharm.2017.07.029>.

2.6. Measurement of von Frey threshold

Mechanical allodynia to a punctate stimulus was determined with a slight modification of a previously described method (Chaplan et al., 1994). Briefly, animals were acclimated for 2 h in methacrylate test compartments (7.5 cm wide × 7.5 cm long × 15 cm high) placed on an elevated mesh-bottomed platform, to provide access to the plantar surface of the hindpaws. Plantar stimulation is the standard site of application of von Frey filaments in animals with joint inflammation (e.g. Nieto et al., 2015; Mangione et al., 2016). A logarithmic series of calibrated von Frey monofilaments (Stoelting, Wood Dale, IL, USA), with bending forces that ranged from 0.02 to 1.4 g, were applied using the up–down paradigm, starting with the 0.6 g filament. Filaments were applied two times for 2–3 s, with inter-application intervals of at least 30 s to avoid sensitization to the mechanical stimuli. The response to the filament was considered positive if immediate licking/biting, flinching or rapid withdrawal of the stimulated paw was observed. As for grip strength determinations, basal values were recorded for each animal as the average of two determinations on different days, before the administration of CFA or saline. This value was considered 100% of the von Frey threshold and used as a reference for subsequent determinations.

2.7. Locomotor activity measurements

Ambulatory locomotion was measured with an infrared detector (Med associated Inc., St. Albans, VT, USA) equipped with 48 infrared photocell emitters and detectors, according to a previously described method (Sánchez-Fernández et al., 2013) with slight modifications. Briefly, animals were placed individually in transparent evaluation chambers (27.5 cm wide × 27.5 cm long × 20 cm high) and the distance traveled (horizontal activity) and the number of rears (vertical activity) were recorded during 30 min. Animals were tested only once to avoid habituation to the evaluation chambers, which markedly decreases their locomotor activity. All experiments were done in a sound-proofed room with dim light (7–10 lux; Luxometer SM700, Milwaukee 4 Electronics Kft., Szeged, Hungary). No experimenters were present in the testing room during the evaluation period.

2.8. Histology

Mice were anesthetized with isoflurane (IsoVet[®], B. Braun, Barcelona, Spain) and perfused intracardially with 20 mL saline followed by 4% formaldehyde solution. After perfusion, the paws were dissected and fixed with 10% buffered formalin for 48 h at room temperature. Joints were then decalcified in Anne Morse solution (50 mL formic acid and 50 mL 20% sodium citrate) at room temperature for 14 days. Next, they were sectioned longitudinally, dehydrated with alcohol, and embedded in paraffin. We used a similar protocol to stain paw pad samples, but obtained the tissue with a 3-mm punch, and omitted the decalcification step. Tissue sections (7–10 μm) were stained with hematoxylin and eosin. Images were acquired with a Nikon Eclipse 50i microscope equipped with a DS-Ri1 camera.

2.9. Immunohistochemistry

Mice were transcardially perfused as above, and the L4 dorsal root ganglion and the spinal cord lumbar enlargement were dissected, postfixed, dehydrated and embedded in paraffin using standard procedures. Tissue sections (5 μm) were deparaffinized in xylol (Panreac Quimica, Castellar del Vallès, Spain) and rehydrated. Antigens were retrieved by steam heating with 1% citrate buffer. Sections were incubated for 1 h in blocking solution (5% normal donkey serum, 0.3% Triton X-100, 0.1% Tween 20 in Tris buffer solution). Then the slices were incubated for 1 h at room temperature with a goat anti-TRPV1 antibody (sc-12498, 1:100, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) in blocking solution. After incubation, the sections were washed three times for 10 min and incubated for 1 h with the secondary donkey anti-goat Alexa Fluor-488 antibody (A11055, 1:500, Life Technologies, Alcobendas, Spain) and a conjugated mouse anti-NeuN antibody (MAB377A5, 1:500, Merck Millipore, Madrid, Spain). The slices were then washed three times for 10 min and mounted with ProLong[®] Gold Antifade Mountant (Life Technologies, Alcobendas, Spain). Images were acquired with a confocal laser-scanning microscope (Model A1, Nikon Instruments Europe BV, Amsterdam, Netherlands).

2.10. Data analysis

The data were analyzed with the SigmaPlot 12.0 program (Systat Software Inc., San Jose, CA, USA). Two-way repeated-measures analysis of variance (ANOVA) or one-way ANOVA were used depending on the experiment. The Student–Newman–Keuls post-test was used in all cases. The differences between means were considered significant when the *P* value was below 0.05.

3. Results

3.1. Behavioral phenotyping of mice with CFA-induced joint inflammation: grip strength, mechanical allodynia, body weight and locomotor activity

The administration of 20 or 30 μL/ankle of CFA induced volume-dependent decreases in hindlimb grip strength (about 35% for 20 μL and 55% for 30 μL) during the first 3 days after the induction of inflammation. In both dose groups, grip strength values returned to normal levels, although the recovery period was longer in animals given the higher volume of CFA (Fig. 1A). Untreated (naïve) animals and mice treated periarticularly with saline (30 μL/ankle) did not show significant changes in grip strength at any time-point tested for up to 21 days (Fig. 1A).

CFA induced persistent ankle swelling that lasted for at least 21 days, which was also dependent on the volume administered.

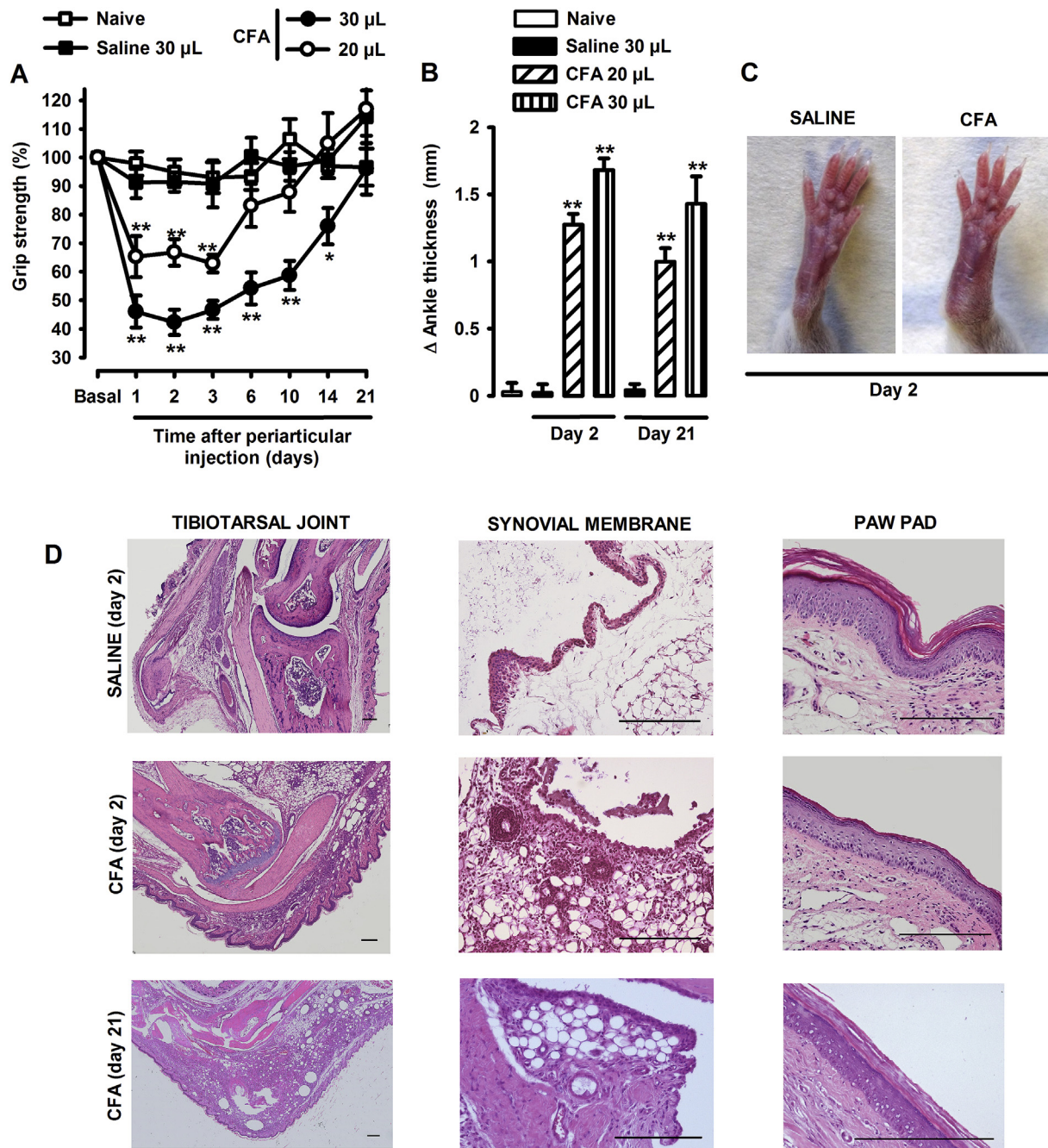


Fig. 1. Grip strength deficits in mice given periarticular injections of CFA were accompanied by edema and localized immune infiltrate. (A) Time-course of hindlimb grip strength in animals treated with CFA (20 or 30 µL/ankle), compared to naïve mice and animals treated with saline (30 µL/ankle). (B) Increase in ankle thickness after the administration of CFA or saline (20 or 30 µL/ankle). (C) Representative pictures of mice treated periarticularly with 30 µL/ankle of saline or CFA (left and right panels, respectively). (D) Representative photomicrographs of hematoxylin and eosin-stained tibiotarsal joint, synovial membrane and paw pad in mice after the administration of saline or CFA (30 µL/ankle); the scale bars represent 200 µm in all panels. Values are the mean \pm SEM (10–12 animals per group). Statistically significant differences in (A) and (B): ** $P < 0.01$ versus the values from naïve animals (two-way repeated measures ANOVA in (A) and one-way ANOVA in (B) both followed by Student–Newman–Keuls test). There were no statistically significant differences between values from naïve animals and saline-treated mice. Data for ankle swelling and histological analyses were obtained from the same mice.

Animals treated with 30 µL/ankle of CFA had thicker ankles than those given 20 µL, whereas those treated with saline (30 µL/ankle) showed no significant increase in ankle thickness (Fig. 1B). We used 30 µL CFA (or saline)/ankle in the rest of the experiments. Macroscopically, saline-treated animals showed no signs of paw inflammation (Fig. 1C, left panel). However, CFA-treated mice had prominent inflammation that appeared to be restricted to the administration site (ankle joint) and nearby areas, whereas the paw

pad did not appear to be affected (Fig. 1C right panel). Histological examination of the tibiotarsal joint, synovial membrane or paw pad from mice 2 days after the administration of saline did not disclose any histological anomalies (Fig. 1D upper panels); however, 2 days after CFA administration we found massive periarticular immune infiltrate which extended to the heel, as well as an inflammatory process in the synovial membrane characterized by prominent immune infiltrate accompanied by intraarticular exudate, but no

histological alterations in the paw pad (Fig. 1D, middle panels). Twenty-one days after CFA administration, the animals still showed marked immune infiltration in the heel and periarticular structures, but the synovial membrane showed no immune infiltrate, and we did not observe appreciable intraarticular exudate, and the paw pad remained unaffected (Fig. 1D, lower panels).

We then measured the mechanical threshold in an inflamed (heel) and in a noninflamed area (pad) of the paw. Mice given CFA showed a marked decrease in the von Frey threshold in the heel, denoting the presence of tactile allodynia, and the decrease was maintained throughout the time-course of study (21 days) (Fig. 2A). Joint inflammation did not induce alterations in the mechanical threshold in the paw pad (Fig. 2A), indicating that the sensory alterations appeared to be restricted to the inflamed area. Control mice treated periarticularly with saline showed no alterations in their mechanical thresholds in either the heel or the paw pad (Fig. 2A).

We also determined whether joint inflammation under our experimental conditions induced other alterations such as changes in body weight or locomotion. Animals with induced inflammation showed no changes in body weight in comparison to saline-treated mice (Fig. 2B). In addition, CFA administration did not induce alterations in either vertical or horizontal locomotor activity in comparison to control mice (Fig. 2C). Therefore, this indicates that although CFA administration induced an apparent decrease in grip strength and mechanical threshold, it did not significantly affect the animals' general state or general mobility.

3.2. Effects of conventional oral analgesics on inflammation-induced grip strength deficits and tactile allodynia

To test whether grip strength deficits were related to pain, we evaluated the effects of conventional analgesics. Oral administration of the NSAIDs ibuprofen and celecoxib, as well as acetaminophen, produced a dose-dependent increase in grip strength in animals with joint inflammation (Fig. 3A, B and C, respectively). The maximum effect of all three drugs peaked at 90 min after administration, but even at this time-point grip strength in mice with induced inflammation failed to fully recover basal values. Subsequently, the increase in grip strength in animals with joint

inflammation induced by the NSAIDs or acetaminophen gradually reverted (Fig. 3A, B and C). We also tested the effects of oral administration of the opioids tramadol and oxycodone (Fig. 4A and B, respectively). These drugs induced a rapid recovery of physical function peaking at 45 min after administration, which decreased gradually with time. In contrast to the effects of NSAIDs or acetaminophen, both tramadol and oxycodone induced full recovery from grip strength deficits in mice with joint inflammation (Fig. 4A and B, respectively). None of these analgesics was able to modify grip strength values in animals without inflammation, even when administered at doses that had maximal effects in mice with induced inflammation (Fig. 3A, B and C and Fig. 4A and B). These results suggest that the analgesics tested improved grip strength deficits by pain-specific effects rather than by altering normal motor function.

We then constructed the dose-response curves of drug effects at their time of maximum effect (90 min for the NSAIDs and acetaminophen, and 45 min for the opioid drugs), to facilitate comparisons of the effects of different drugs on the recovery of grip strength in mice with joint inflammation. Maximal effects of ibuprofen, celecoxib and acetaminophen led to a recovery of grip strength of about 60% of pre-inflammation values, whereas in animals treated with the opioids oxycodone or tramadol, grip strength recovered to approximately 90% of control values (Fig. 5A).

We then tested the effects of all drugs on tactile allodynia in mice with induced inflammation. Doses of ibuprofen, celecoxib, acetaminophen or tramadol that induced ≈60% recovery of grip strength (40 mg/kg, 80 mg/kg, 160 mg/kg and 20 mg/kg, respectively) were devoid of effect on tactile allodynia at any time-point tested between 45 and 240 min (Suppl Fig. 1A for NSAIDs and acetaminophen, and Suppl Fig. 1B for tramadol). On the other hand, oxycodone 4 mg/kg induced a modest but significant amelioration of mechanical allodynia at 45 min (Suppl Fig. 1B), which coincided with its peak effect on grip strength deficits (Fig. 4B). This effect rapidly disappeared, which is in contrast to the longer duration of its effects on grip strength deficits, which lasted for 90 min (Suppl Fig. 1B and Fig. 4B). To facilitate comparisons of the effects of these drugs on the recovery of grip strength and mechanical allodynia only the time-point of 90 min for the NSAIDs and acetaminophen,

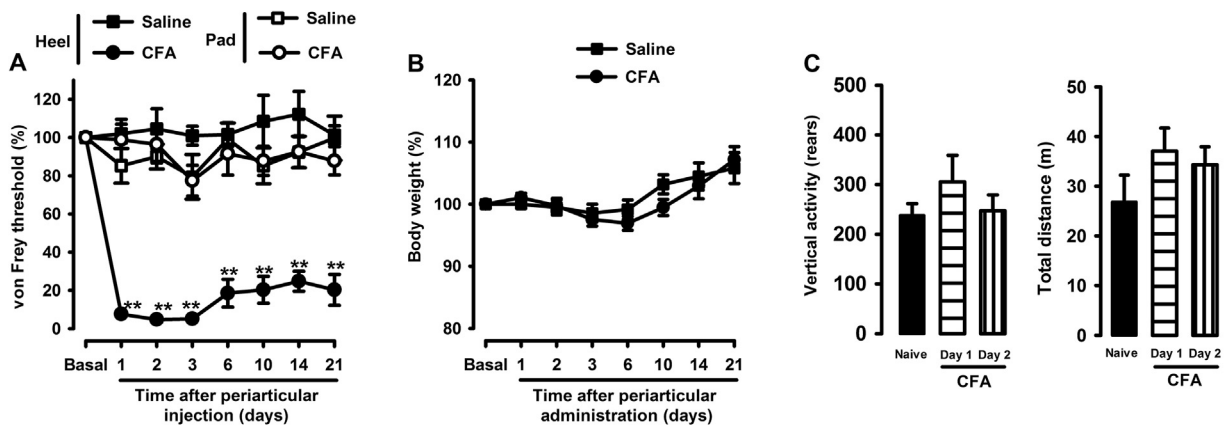


Fig. 2. Mechanical allodynia in mice given periarticular injections of CFA did not parallel alterations in body weight or locomotor activity. (A) von Frey threshold in the heel and the paw pad in mice treated periarticularly with CFA or saline. (B) Time-course of body weight after the administration of CFA or saline. (C) Vertical (rears) and horizontal (distance traveled) locomotor activity of naïve mice and CFA-treated animals, 1 or 2 days after the induction of periarticular inflammation. A volume of 30 µL/ankle was used in all experiments. Values are the mean ± SEM (10–12 animals per group). Statistically significant differences in: (A) $**P < 0.01$ between the values from mice with and without inflammation stimulated in the heel at each time-point (two-way repeated measures ANOVA followed by Student–Newman–Keuls test); (B) no statistically significant differences between values from saline- or CFA-treated animals at any time-point tested (two-way repeated measures ANOVA); (C) no statistically significant differences between values from naïve- or CFA-treated animals (one-way ANOVA).

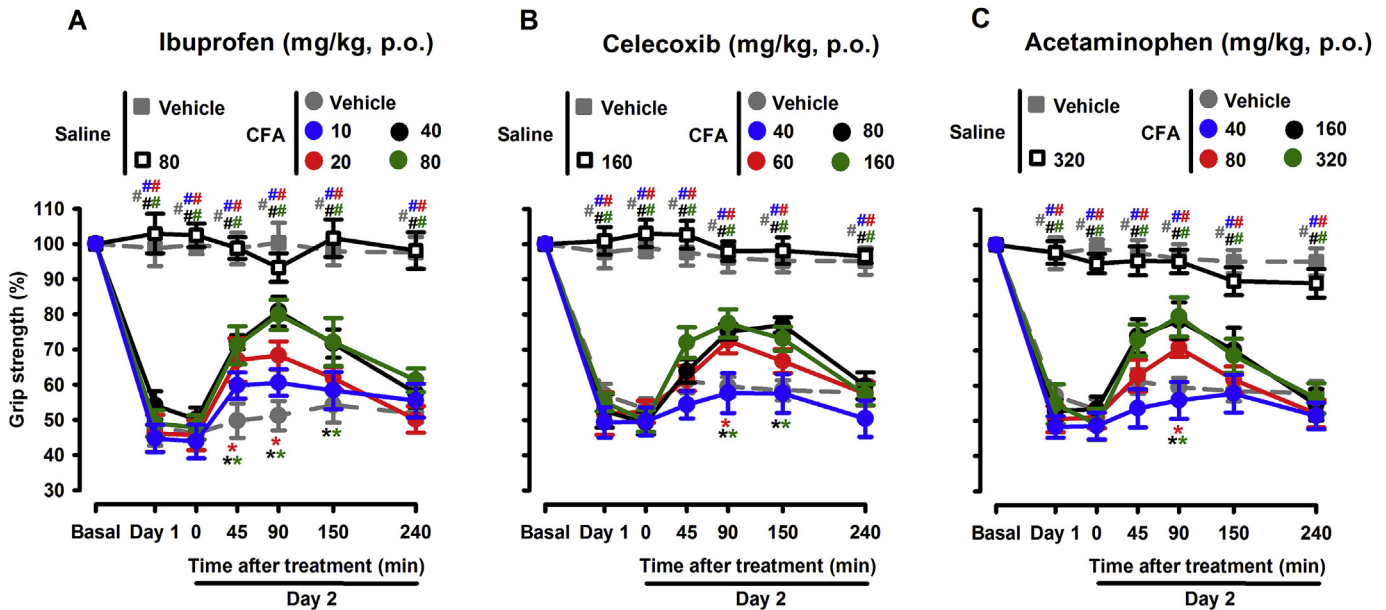


Fig. 3. Effects of oral NSAIDs and acetaminophen on grip strength deficits induced by the periarticular administration of CFA. Ibuprofen (A), celecoxib (B), acetaminophen (C) or their solvents (HPMC for ibuprofen, and 1% Tween 80 in HPMC for celecoxib and acetaminophen) were given orally (p.o.) 2 days after the administration of CFA or saline (30 μ L/ankle). Values are the mean \pm SEM (10–12 animals per group). Statistically significant differences in (A), (B) and (C): * $P < 0.05$ between the values from mice with inflammation treated with the drug or solvent at each time-point; # $P < 0.05$ between the values from mice without inflammation treated with the drug solvents and mice with inflammation at each time-point (two-way repeated measures ANOVA followed by Student–Newman–Keuls test). For clarity, significances of $P < 0.01$ are not labeled with double symbols. There were no significant differences between mice without inflammation treated with the drugs or their solvents at any time-point tested.

and 45 min for the opioid drugs, are shown in Fig. 5B. When we doubled the doses of these drugs all of them were then able to ameliorate tactile allodynia (Fig. 5B). Therefore, although all analgesics tested were able to improve both physical function and tactile allodynia, grip strength was a more sensitive indicator of drug-induced analgesia than tactile allodynia.

3.3. Effects of the muscle relaxant baclofen on inflammation-induced grip strength deficits and mechanical allodynia

To determine the impact of motor impairment on grip strength and tactile allodynia, we tested the effects of the muscle relaxant baclofen. This drug induced opposite effects on grip strength and

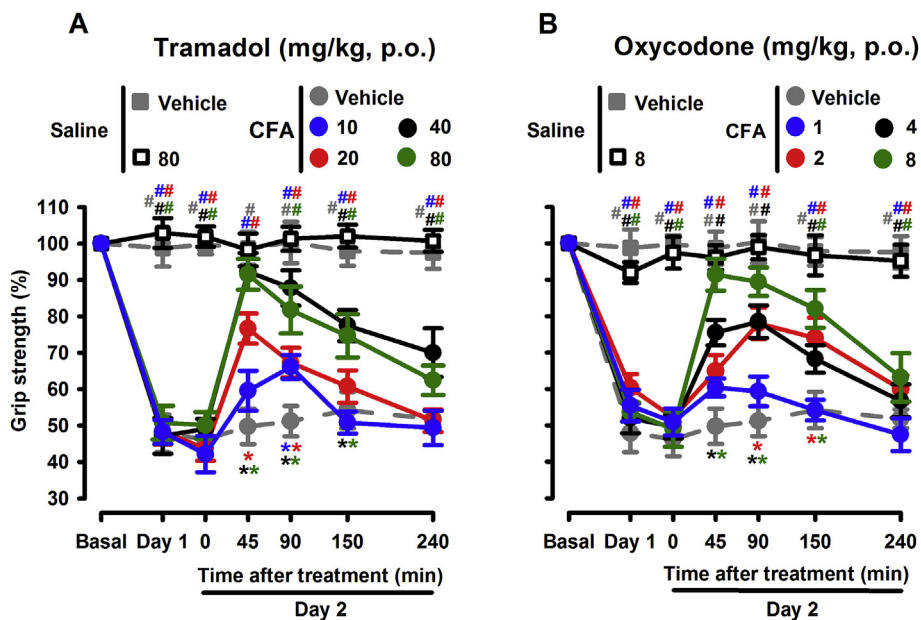


Fig. 4. Effects of oral opioids on grip strength deficits induced by the periarticular administration of CFA. Tramadol (A), oxycodone (B) or their solvent (HPMC) were administered orally (p.o.) 2 days after the administration of CFA or saline (30 μ L/ankle). Values are the mean \pm SEM (10–12 animals per group). Statistically significant differences in (A) and (B): * $P < 0.05$ between the values from mice with inflammation treated with the drug or solvent at each time-point; # $P < 0.05$ between the values from mice without inflammation treated with the drug solvents and mice with inflammation at each time-point (two-way repeated measures ANOVA followed by Student–Newman–Keuls test). For clarity, significances of $P < 0.01$ are not labeled with double symbols. There were no significant differences between mice without inflammation treated with the drugs or their solvents at any time-point tested.

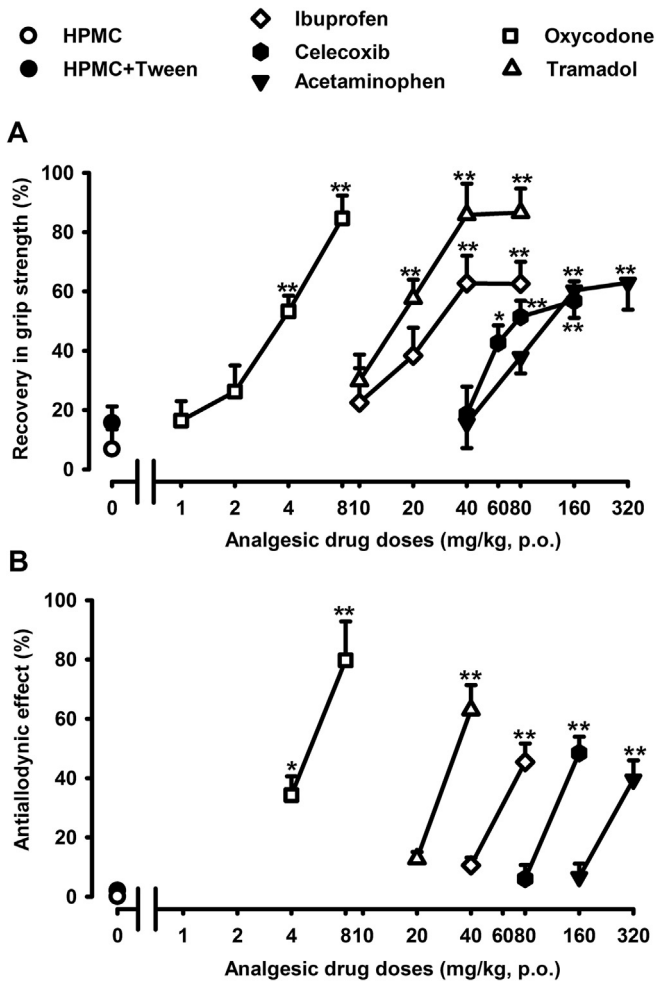


Fig. 5. Comparison of the effects induced by the oral administration of analgesic drugs on grip strength deficits and tactile allodynia in mice treated periarticularly with CFA. (A) Effects of analgesic drugs on grip strength deficits and (B) on the von Frey threshold in the heel of animals with inflammation. In all cases, drugs or their solvents were administered orally (p.o.) 2 days after CFA administration (30 μ L/ankle). HPMC was used as the solvent for ibuprofen, tramadol and oxycodone (white symbols), and 1% Tween 80 in HPMC for celecoxib and acetaminophen (black symbols). Values are the mean \pm SEM (10–12 animals per group). Data are expressed as the percent recovery of grip strength or percent antiallodynic effect, considering as 0% effect the values obtained immediately before drug administration, and a 100% effect the values obtained before CFA injection in each individual mouse. Values were obtained 45 min after tramadol or oxycodone administration, or 90 min after ibuprofen, celecoxib, or acetaminophen administration. Statistically significant differences in (A) and (B): * $P < 0.05$, ** $P < 0.01$ between the values from mice with inflammation treated with the drugs or their solvents (one-way ANOVA followed by Student–Newman–Keuls test).

tactile allodynia: it dose-dependently increased the von Frey threshold in mice with inflammation, inducing a marked antiallodynic-like effect (to an extent similar to oxycodone), and did not ameliorate grip strength deficits, but induced a parallel decrease in this functional measure (Fig. 6). In addition, the active doses of baclofen also decreased grip strength in noninjured animals (data not shown).

3.4. Effects of the TRP antagonist ruthenium red on inflammation-induced grip strength deficits and mechanical allodynia

We also evaluated whether grip strength deficits and mechanical allodynia during inflammation were sensitive to the TRP antagonist ruthenium red. The systemic administration of this

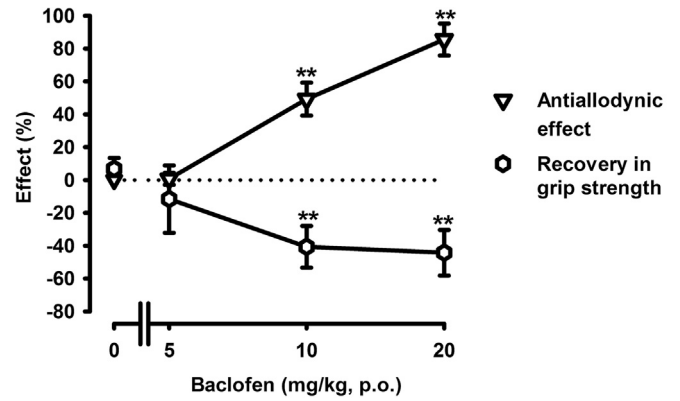


Fig. 6. Comparison of the effects induced by the oral administration of baclofen on grip strength deficits and tactile allodynia in mice treated periarticularly with CFA. Baclofen or its solvent (HPMC) was administered orally (p.o.) 2 days after CFA administration (30 μ L/ankle). Values are the mean \pm SEM (10–12 animals per group). The data are expressed as the percent recovery of grip strength or percent antiallodynic effect, considering the values obtained immediately before drug administration as 0%, and the values obtained before CFA injection in each individual mouse as 100%. All values were obtained 45 min after the administration of baclofen or its solvent. Statistically significant differences: * $P < 0.05$, ** $P < 0.01$ between the values from mice with inflammation treated with the drugs or their solvents (one-way ANOVA followed by Student–Newman–Keuls test).

compound (1–2 mg/kg) did not affect grip strength in injured or noninjured mice at any time-point tested between 30 and 180 min (Suppl. Fig. 2). For clarity, only the data for 2 mg/kg 30 min post-administration are shown in Fig. 7A. However, ruthenium red (administered 30 min before the behavioral evaluation) was able to abolish, in a dose-dependent manner, mechanical allodynia in mice with inflammation but without affecting the mechanical threshold in noninjured mice (Fig. 7B). We were unable to test a higher dose of ruthenium red (4 mg/kg) because it induced prominent side effects. The differential effects of ruthenium red on grip strength deficits and tactile allodynia suggest that their mechanisms differ.

3.5. Contribution of TRPV1-expressing neurons to inflammation-induced grip strength deficits and tactile allodynia

To investigate whether grip strength deficits and mechanical allodynia during inflammation depend on the same type of nociceptive neurons, we compared the effects on grip strength and mechanical threshold of the *in vivo* ablation of TRPV1-expressing neurons. TRPV1 staining is present in the somas of small DRG neurons and in the superficial layers of the spinal cord dorsal horn (see controls in Fig. 8A and B, respectively). After RTX treatment we were unable to detect TRPV1 staining in either the DRG (Fig. 8A) or the spinal cord dorsal horn (Fig. 8B), reflecting the ablation of TRPV1-expressing neurons including their central terminals. The ablation of this nociceptive population did not affect either grip strength or mechanical threshold in noninjured mice (Fig. 8C and D, respectively). However, it was able to prevent the development of mechanical allodynia in mice with inflammation (Fig. 8D), although it had no effect on their grip strength deficits (Fig. 8C). Therefore, grip strength deficits and the decrease in the von Frey threshold during joint inflammation involve the participation of different populations of primary afferents.

4. Discussion

In this study we show that in mice with experimentally-induced joint inflammation, grip strength decreased markedly and for a

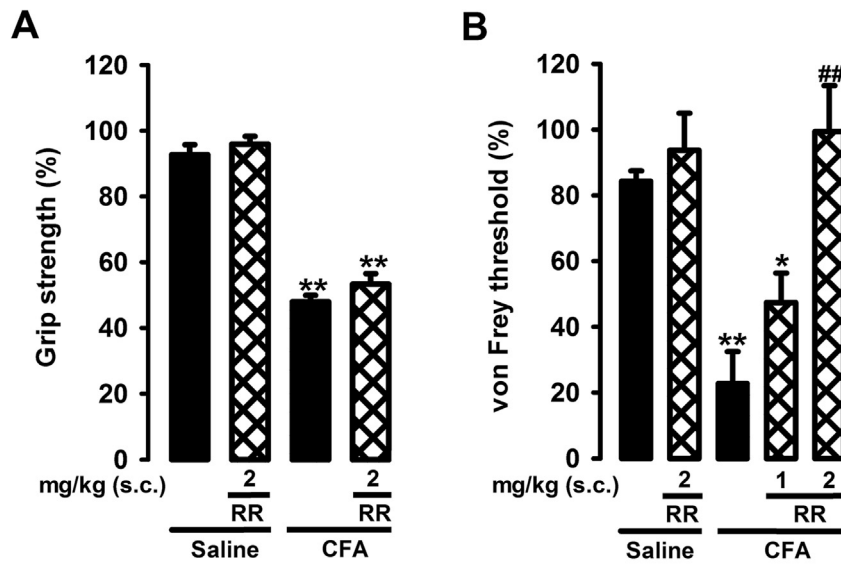


Fig. 7. Effects of the subcutaneous administration of ruthenium red on grip strength deficits and von Frey threshold in mice treated periarticularly with CFA. (A) Absence of effect of ruthenium red (RR) on grip strength deficits induced by CFA. (B) RR, administered 30 min before the behavioral evaluation, attenuated the decrease in von Frey threshold induced by CFA. RR or its solvent (saline) was administered subcutaneously (s.c.) 2 days after CFA administration (30 μ L/ankle). Values are the mean \pm SEM (10–12 animals per group). Statistically significant differences in (A) and (B): ** $P < 0.01$ between the values from mice with and without inflammation treated with ruthenium red or its solvent; ## $P < 0.01$ between the values from mice with inflammation treated with ruthenium red or its solvent (one-way ANOVA followed by Student–Newman–Keuls test).

prolonged period. Grip strength deficit and mechanical allodynia (measured with von Frey filaments) in the inflamed area differed in both their time-courses of evolution and in their sensitivity to conventional analgesics. In addition, we show that although tactile allodynia was abolished by ruthenium red or by the ablation of TRPV1-expressing neurons, deficits in grip strength in mice with joint inflammation were not.

The time-courses of recovery from grip strength deficits and mechanical allodynia differed, as the latter persisted longer than the functional deficit. The different time-courses of evolution in these two outcomes indicate that pronounced tactile hypersensitivity in the inflamed area does not necessarily imply a significant alteration in physical function. We show that the periarticular administration of CFA in the ankle joint induced prominent, long-lasting ankle swelling. However, this sustained ankle swelling was accompanied by histological alterations which differed in duration. Both the inflammatory process in the synovial membrane (i.e. immune infiltrate and intraarticular exudates) and grip strength deficits were prominent 2 days after inflammation was induced, whereas they both became attenuated 21 days after CFA injection. These results suggest that synovial membrane alterations may be related to the functional deficits observed, and in this connection, synovitis has been strongly linked to both joint dysfunction and pain in human studies (reviewed in Rice et al., 2015; Scanzello and Goldring, 2012; Scott et al., 2000). Under our experimental conditions, the immune infiltrate in mice with inflammation extended to the heel and was prominent throughout the entire study period, as was mechanical allodynia in this area of the paw (in contrast to the recovery of the synovial membrane and grip strength). The persistent immune infiltrate in the heel may contribute to the long-lasting tactile hypersensitivity we detected in our mice, as it is known that immune cells play a pivotal role in inflammatory cutaneous allodynia (Ghasemlou et al., 2015). These results suggest that the histological alterations which drive functional deficits and tactile allodynia may differ. We did not detect either observable histological or sensory alterations in the paw pad of mice with periarticular CFA-induced inflammation. Of note regarding this observation is that mice gripped the metal bar

connected to the force transducer with this part of the paw, and therefore, the deficits seen in grip strength in animals with inflammation cannot be ascribed to alterations in paw pad sensitivity (as mice do not develop allodynia in this area) but is instead likely to be the result of movement or tension in the inflamed joint during gripping.

Pain states can curtail body weight gain of rodents (Blackburn-Munro, 2004) and their exploratory activity, particularly their vertical activity (rearing) when the lower limbs are injured (reviewed by Cobos and Portillo-Salido, 2013). We found that mice with joint inflammation, despite their marked grip strength deficits, did not show alterations in body weight gain or exploratory activity. These results indicate that grip strength deficits are a more sensitive indicator of functional alterations than other pain-related outcomes.

To test whether the decrease in grip strength was related to pain, we investigated the effects of orally administered analgesics used for clinical treatment in humans. The NSAIDs ibuprofen and celecoxib, as well as acetaminophen, induced a significant but limited recovery from grip strength deficits. However, the opioids tramadol and oxycodone were able to completely reverse the functional deficit induced by joint inflammation. The higher efficacy of opioids in comparison to NSAIDs or acetaminophen in the recovery of grip strength is in agreement with the analgesic efficacy of these drugs in human patients, according to the WHO analgesic ladder (Sarzi-Puttini et al., 2012); therefore the alterations in grip strength during joint inflammation in our experimental animals are largely attributable to pain. This link between pain and grip strength deficits in mice agrees with the known correlation between pain and disability according to the same outcome measure in patients with joint pain (e.g. Fraser et al., 1999; Overend et al., 1999). Sensitivity to analgesic treatment differed between grip strength recovery and mechanical allodynia: the former was a more sensitive indicator of the effects of analgesic drugs than the latter. To the best of our knowledge, the different sensitivities of tactile hypersensitivity and grip strength deficits to the effects of drugs have not been explored in earlier research that used grip strength as a pain outcome measure. The greater sensitivity of grip strength

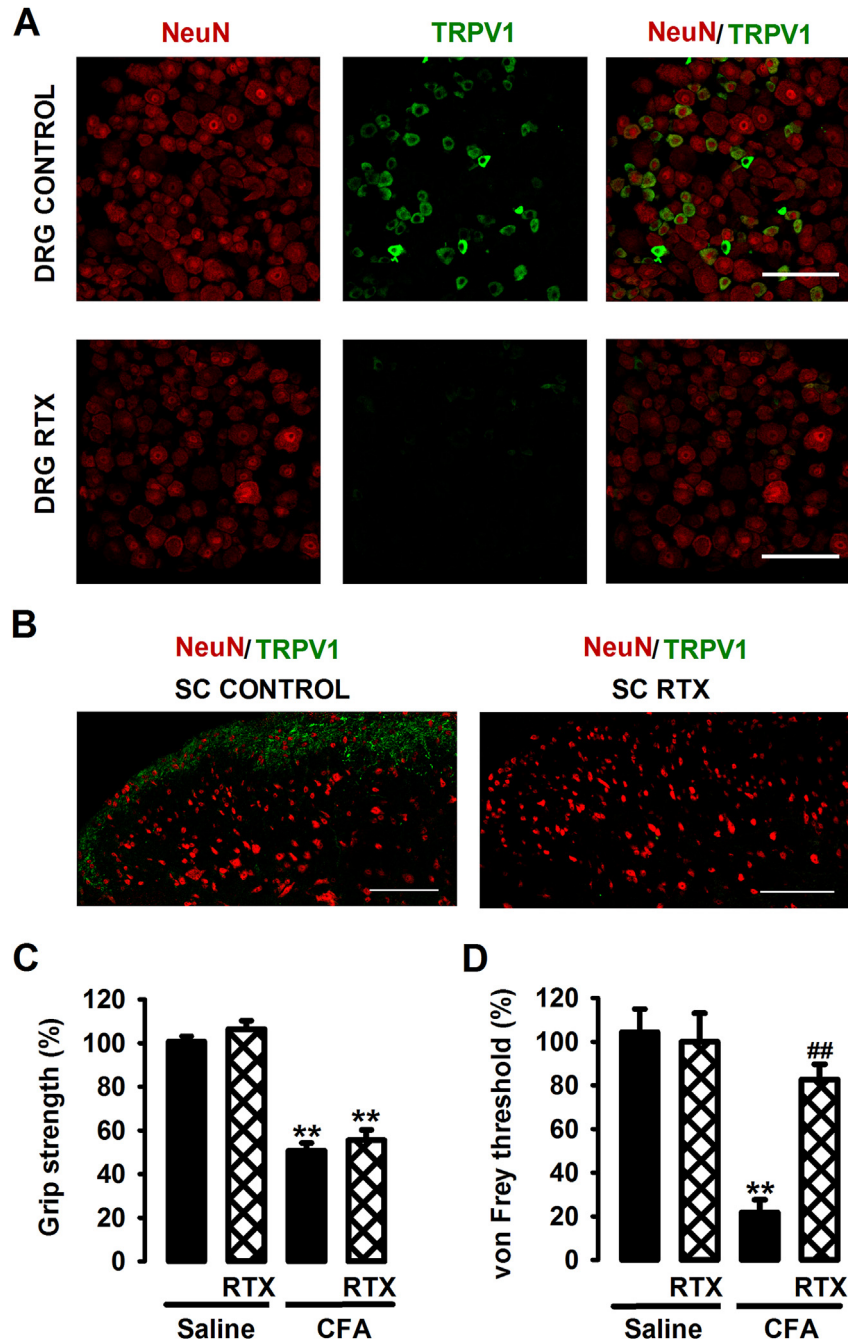


Fig. 8. Differential effects of the ablation of TRPV1-expressing neurons on grip strength deficits and von Frey threshold in mice treated periarticularly with CFA. (A) and (B) Double labeling of NeuN (red) and TRPV1 (green) in the L4 dorsal root ganglion (DRG) and in the spinal cord (SC) lumbar enlargement from CFA-treated mice. Top panels: samples from saline-treated mice (control). Bottom panels: samples from mice treated with resiniferatoxin (RTX). The scale bars represent 100 μ m. (C) Absence of effect of RTX on grip strength deficits induced by CFA. (D) RTX attenuated the decrease in von Frey threshold induced by CFA. Sample collection and behavioral testing were done 2 days after the periarticular administration of CFA or saline (30 μ L/ankle), in mice treated with RTX (50 μ g/kg) or its solvent. Values are the mean \pm SEM (10–12 animals per group). Statistically significant differences in (C) and (D): ** $P < 0.01$ between the values from mice with and without inflammation treated with RTX or its solvent; ## $P < 0.01$ between the values from mice with inflammation treated with RTX or its solvent (one-way ANOVA followed by Student–Newman–Keuls test). The same mice were used for the behavioral and immunofluorescence assays.

compared to tactile allodynia in reflecting the effects of drug-induced analgesia is in agreement with previous studies with other measures of physical functioning in rodents, such as changes in weight bearing in the injured paw, exploratory locomotion, burrowing behavior or wheel running (Andrews et al., 2012; Cobos et al., 2012; Huntjens et al., 2009; Matson et al., 2007). Therefore, the greater sensitivity to drug-induced analgesia may be an

inherent quality of these types of more “natural” pain measures, as previously suggested (Cobos and Portillo-Salido, 2013; De la Puente et al., 2015). This might be particularly relevant for analgesic drug discovery, because many new potentially interesting compounds are discarded based on their lack of efficacy on tactile allodynia, although they might ameliorate functional measures of pain which currently are not routinely evaluated.

Importantly, none of the analgesics we tested modified grip strength in animals without inflammation, and therefore the recovery of grip strength in mice with joint inflammation was not due to nonspecific drug effects on normal grip strength.

Drugs may inhibit pain-like reflexes due to sedative and motor effects, resulting in false-positive results. Here we show that baclofen induced a marked increase in the von Frey threshold in mice with inflammation, and its effect was similar to that of the third-step opioid oxycodone. However, we found that “antiallodynic” doses of this drug also decreased grip strength in mice with and without inflammation. This suggests that the effects induced by baclofen on inflammatory mechanical allodynia are due to this drug’s known motor impairment and sedative effects (Dario and Tomei, 2004) rather than to a true analgesic effect. Therefore, grip strength and von Frey threshold are not affected in the same way by the same confounders. Whereas drug-induced motor impairment might lead to a false analgesic-like effect in the von Frey test, it would not be expected to have this effect in tests of grip strength as a surrogate measure of pain. For analgesic drug discovery it is essential to determine whether drugs induce signs of toxicity. Grip strength has been used in humans to test drug-induced toxicity (Savilampi et al., 2014) it is classically used to assess neurotoxicity in rodents (Meyer et al., 1979) and is even included in the Irwin screen (Irwin, 1968; Mattsson et al., 1996) which is ingrained in the pharmaceutical industry as the first tier of preclinical testing to detect drug-induced neurotoxic effects (Moser, 2011). Therefore, this outcome measure can be used to detect both drug-induced analgesia and toxicity, which is undoubtedly advantageous to determine the therapeutic index of drugs being tested during preclinical development.

The different responses of grip strength deficits and tactile allodynia to analgesic treatment, together with the previously noted differences in their time-courses, support the notion that different mechanisms are involved in the appearance of mechanical allodynia and joint pain-induced functional disability. In fact, we show that the nonselective TRP antagonist ruthenium red was able to abolish inflammatory tactile allodynia but without ameliorating grip strength deficits. Our results using ruthenium red agree with its previously reported effects on tactile allodynia from diverse etiology (Cui et al., 2014; Qu et al., 2016; Shinoda et al., 2008). Ruthenium red is a widely used TRP antagonist (e.g. St Pierre et al., 2009), although it also blocks other channels, including the mechanosensitive Piezo channels (Coste et al., 2012). Therefore, although our results indicate that tactile allodynia and grip strength deficits are not affected in the same way by this compound (and hence suggest that their mechanisms differ), this does not definitively link TRP channels to the effects we observed.

We then targeted the function of TRPV1+ neurons, which are known to express several TRP channels (Julius, 2013). In our study the ablation of these TRPV1-expressing sensory neurons did not alter the normal mechanical threshold, but completely attenuated tactile allodynia, which is consistent with the known pivotal role of TRPV1 neurons in cutaneous pain hypersensitivity during inflammatory arthritis (Borbély et al., 2015). However, we found no effect on joint inflammation-induced grip strength deficits, indicating that TRPV1-expressing neurons are not responsible for the functional deficits observed. Grip strength has been previously used as an indicator of pain-induced disability in osteoarthritic pain (Chandran et al., 2009; Honore et al., 2009). It has been reported that TRPV1 antagonism is able to ameliorate grip strength deficits in rodents with experimental osteoarthritis (Honore et al., 2009). Osteoarthritis has been classically considered a “noninflammatory arthritis” (e.g. Haroon et al., 2016), and although it is currently believed that osteoarthritis has an inflammatory component (Sokolove and Lepus, 2013), this is not as prominent as in our CFA-

treated mice. Therefore, the differences between our findings and previous reports might be due to differences in the type of joint pain explored.

TRPV1 is present in nearly all unmyelinated (C-type) peptidergic neurons in the mouse DRG (Cavanaugh et al., 2011), but is almost absent from A-neurons and virtually absent from C-nonpeptidergic nociceptors (e.g. Cavanaugh et al., 2011; Niiyama et al., 2007). Therefore, other nociceptive neurons different from C-peptidergic nociceptors, such as A δ or C-nonpeptidergic neurons (which express little or no TRPV1) (e.g. Niiyama et al., 2007), might contribute to grip strength deficits during joint inflammation. An alternative explanation is that joint tissues are also innervated by proprioceptors, which under painful conditions might lead to pain via central mechanisms (e.g. Mapp, 1995). Regardless of the exact mechanism involved in grip strength deficits during joint inflammation, the differential effects of ruthenium red and the ablation of TRPV1-expressing neurons on tactile allodynia and functional disability during joint inflammation strongly support the notion that their biological mechanisms differ.

5. Conclusions

We conclude that monitoring grip strength deficits during joint inflammation is a reliable measure of inflammatory joint pain in rodents, as it is known to be in humans. Grip strength can be used to characterize the efficacy of analgesic treatment as well as the appearance or severity of drug-induced neurotoxic effects (a prominent confounder in preclinical analgesia development), and can be readily used by academic laboratories as well as by the pharmaceutical industry. The neurobiological mechanisms involved in grip strength deficits and tactile allodynia during inflammation differ, and therefore the results obtained with measures of cutaneous hypersensitivity alone in preclinical drug testing cannot be directly extrapolated to inflammatory joint pain-induced functional impairment. The evaluation of grip strength deficits holds potential to improve the reliability of preclinical evaluations of new pain targets and candidate analgesics by providing a measure in rodents of a parameter widely used in the clinical practice.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.neuropharm.2017.07.029>.

References

Andrews, N., Legg, E., Lisak, D., Issop, Y., Richardson, D., Harper, S., Pheby, T.,

- Huang, W., Burgess, G., Machin, I., Rice, A.S., 2012. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *Eur. J. Pain* 16, 485–495.
- Barrett, J.E., 2015. The pain of pain: challenges of animal behavior models. *Eur. J. Pharmacol.* 753, 183–190.
- Bennett, M., 2001. The LANS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. *Pain* 92, 147–157.
- Bijlsma, J.W., Huber-Bruning, O., Thijssen, J.H., 1987. Effect of oestrogen treatment on clinical and laboratory manifestations of rheumatoid arthritis. *Ann. Rheum. Dis.* 46, 777–779.
- Blackburn-Munro, G., 2004. Pain-like behaviours in animals - how human are they? *Trends Pharmacol. Sci.* 25, 299–305.
- Borbély, E., Botz, B., Bölskei, K., Kenyér, T., Kereskai, L., Kiss, T., Szolcsányi, J., Pintér, E., Csepregi, J.Z., Mócsai, A., Helyes, Z., 2015. Capsaicin-sensitive sensory nerves exert complex regulatory functions in the serum-transfer mouse model of autoimmune arthritis. *Brain Behav. Immun.* 45, 50–59.
- Bouhassira, D., Attal, N., Alchaar, H., Boureau, F., Brochet, B., Bruxelle, J., Cunin, G., Fermanian, J., Ginies, P., Grun-Overdyking, A., Jafari-Schluep, H., Lanteri-Minet, M., Laurent, B., Mick, G., Serrie, A., Valade, D., Vicaut, E., 2005. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* 114, 29–36.
- Callahan, L.F., Brooks, R.H., Summey, J.A., Pincus, T., 1987. Quantitative pain assessment for routine care of rheumatoid arthritis patients, using a pain scale based on activities of daily living and a visual analog pain scale. *Arthritis Rheum.* 30, 630–636.
- Cavanaugh, D.J., Chesler, A.T., Braz, J.M., Shah, N.M., Julius, D., Basbaum, A.I., 2011. Restriction of transient receptor potential vanilloid-1 to the peptidergic subset of primary afferent neurons follows its developmental downregulation in nonpeptidergic neurons. *J. Neurosci.* 31, 10119–10127.
- Chandran, P., Pai, M., Blomme, E.A., Hsieh, G.C., Decker, M.W., Honore, P., 2009. Pharmacological modulation of movement-evoked pain in a rat model of osteoarthritis. *Eur. J. Pharmacol.* 613, 39–45.
- Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* 53, 55–63.
- Chen, Y., Willcockson, H.H., Valtschanoff, J.G., 2009. Vanilloid receptor TRPV1-mediated phosphorylation of ERK in murine adjuvant arthritis. *Osteoarthr. Cartil.* 17, 244–251.
- Cobos, E.J., Portillo-Salido, E., 2013. “Bedside-to-Bench” behavioral outcomes in animal models of pain: beyond the evaluation of reflexes. *Curr. Neuropharmacol.* 11, 560–591.
- Cobos, E.J., Ghasemlou, N., Araldi, D., Segal, D., Duong, K., Woolf, C.J., 2012. Inflammation-induced decrease in voluntary wheel running in mice: a non-reflexive test for evaluating inflammatory pain and analgesia. *Pain* 153, 876–884.
- Coste, B., Xiao, B., Santos, J.S., Syeda, R., Grandl, J., Spencer, K.S., Kim, S.E., Schmidt, M., Mathur, J., Dubin, A.E., Montal, M., Patapoutian, A., 2012. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* 483, 176–181.
- Croci, T., Zariwani, E., 2007. Effect of the cannabinoid CB1 receptor antagonist rimonabant on nociceptive responses and adjuvant-induced arthritis in obese and lean rats. *Br. J. Pharmacol.* 150, 559–566.
- Cui, Y.Y., Xu, H., Wu, H.H., Qi, J., Shi, J., Li, Y.Q., 2014. Spatio-temporal expression and functional involvement of transient receptor potential vanilloid 1 in diabetic mechanical allodynia in rats. *PLoS One* 9, e102052.
- Dario, A., Tomei, G., 2004. A benefit-risk assessment of baclofen in severe spinal spasticity. *Drug Saf.* 27, 799–818.
- De la Puente, B., Romero-Alejo, E., Vela, J.M., Merlos, M., Zamanillo, D., Portillo-Salido, E., 2015. Changes in saccharin preference behavior as a primary outcome to evaluate pain and analgesia in acetic acid-induced visceral pain in mice. *J. Pain Res.* 8, 663–673.
- Percie, Du Sert N., Rice, A.S., 2014. Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain. *Br. J. Pharmacol.* 171, 2951–2963.
- Dworkin, R.H., Turk, D.C., Wyrwich, K.W., Beaton, D., Cleeland, C.S., Farrar, J.T., Haythornthwaite, J.A., Jensen, M.P., Kerns, R.D., Ader, D.N., Brandenburg, N., Burke, L.B., Cella, D., Chandler, J., Cowan, P., Dimitrova, R., Dionne, R., Hertz, S., Jadad, A.R., Katz, N.P., Kehlet, H., Kramer, L.D., Manning, D.C., McCormick, C., McDermott, M.P., McQuay, H.J., Patel, S., Porter, L., Quessy, S., Rappaport, B.A., Rauschkolb, C., Revicki, D.A., Rothman, M., Schmader, K.E., Stacey, B.R., Stauffer, J.W., von Stein, T., White, R.E., Witter, J., Zavisic, S., 2008. Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. *J. Pain* 9, 105–121.
- Fraser, A., Vallow, J., Preston, A., Cooper, R.G., 1999. Predicting 'normal' grip strength for rheumatoid arthritis patients. *Rheumatol. Oxf.* 38, 521–528.
- Ghasemlou, N., Chiu, I.M., Julien, J.P., Woolf, C.J., 2015. CD11b+Ly6G+ myeloid cells mediate mechanical inflammatory pain hypersensitivity. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6808–E6817.
- Goldberg, D.S., McGee, S.J., 2011. Pain as a global public health priority. *BMC Public Health* 11, 770.
- Haroon, M., Rafiq Chaudhry, A.B., Fitzgerald, O., 2016. Higher prevalence of metabolic syndrome in patients with psoriatic arthritis: a comparison with a control group of noninflammatory rheumatologic conditions. *J. Rheumatol.* 43, 463–464.
- Hendiani, J.A., Westlund, K.N., Lawand, N., Goel, N., Lisse, J., McNearney, T., 2003. Mechanical sensation and pain thresholds in patients with chronic arthropathies. *J. Pain* 4, 203–211.
- Honore, P., Chandran, P., Hernandez, G., Gauvin, D.M., Mikusa, J.P., Zhong, C., Joshi, S.K., Ghilardi, J.R., Sevcik, M.A., Fryer, R.M., Segreti, J.A., Banfor, P.N., Marsh, K., Neelands, T., Bayburt, E., Daanen, J.F., Gomtsyan, A., Lee, C.H., Kort, M.E., Reilly, R.M., Surowy, C.S., Kym, P.R., Mantyh, P.W., Sullivan, J.P., Jarvis, M.F., Faltynek, C.R., 2009. Repeated dosing of ABT-102, a potent and selective TRPV1 antagonist, enhances TRPV1-mediated analgesic activity in rodents, but attenuates antagonist-induced hyperthermia. *Pain* 142, 27–35.
- Hsieh, Y.L., Lin, C.L., Chiang, H., Fu, Y.S., Lue, J.H., Hsieh, S.T., 2012. Role of peptidergic nerve terminals in the skin: reversal of thermal sensation by calcitonin gene-related peptide in TRPV1-depleted neuropathy. *PLoS One* 7, e50805.
- Huntjens, D.R., Spalding, D.J., Danhof, M., Della Pasqua, O.E., 2009. Differences in the sensitivity of behavioural measures of pain to the selectivity of cyclo-oxygenase inhibitors. *Eur. J. Pain* 13, 448–457.
- Irwin, S., 1968. Comprehensive observational assessment: ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* 13, 222–257.
- Julius, D., 2013. TRP channels and pain. *Annu. Rev. Cell Dev. Biol.* 29, 355–384.
- Kissin, I., 2010. The development of new analgesics over the past 50 years: a lack of real breakthrough drugs. *Anesth. Analg.* 110, 780–789.
- Lee, Y.C., 2013. Effect and treatment of chronic pain in inflammatory arthritis. *Curr. Rheumatol. Rep.* 15, 300.
- Lolignier, S., Amsalem, M., Maingret, F., Padilla, F., Gabriac, M., Chapuy, E., Eschaler, A., Delmas, P., Busserolles, J., 2011. Nav1.9 channel contributes to mechanical and heat pain hypersensitivity induced by subacute and chronic inflammation. *PLoS One* 6, e23083.
- Mangione, A.S., Obara, I., Maiarú, M., Geranton, S.M., Tassorelli, C., Ferrari, E., Leese, C., Davletov, B., Hunt, S.P., 2016. Nonparalytic botulinum molecules for the control of pain. *Pain* 157, 1045–1055.
- Mao, J., 2009. Translational pain research: achievements and challenges. *J. Pain* 10, 1001–1011.
- Mapp, P.L., 1995. Innervation of the synovium. *Ann. Rheum. Dis.* 54, 398–403.
- Matson, D.J., Broom, D.C., Carson, S.R., Baldassari, J., Kehne, J., Cortright, D.N., 2007. Inflammation-induced reduction of spontaneous activity by adjuvant: a novel model to study the effect of analgesics in rats. *J. Pharmacol. Exp. Ther.* 320, 194–201.
- Mattsson, J.L., Spencer, P.J., Albee, R.R., 1996. A performance standard for clinical and functional observational battery examinations of rats. *J. Am. Coll. Toxicol.* 15, 239–254.
- Meyer, O.A., Tilson, H.A., Byrd, W.C., Riley, M.T., 1979. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. *Neurobehav. Toxicol.* 1, 233–236.
- Mogil, J.S., Crager, S.E., 2004. What should we be measuring in behavioral studies of chronic pain in animals? *Pain* 112, 12–15.
- Moharic, M., Vidmar, G., Burger, H., 2012. Sensitivity and specificity of von Frey's hairs for the diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. *J. Diabetes Complicat.* 26, 319–322.
- Morris, V.H., Cruwys, S.C., Kidd, B.L., 1997. Characterisation of capsaicin-induced mechanical hyperalgesia as a marker for altered nociceptive processing in patients with rheumatoid arthritis. *Pain* 71, 179–186.
- Moser, V.C., 2011. Functional assays for neurotoxicity testing. *Toxicol. Pathol.* 39, 36–45.
- Negus, S.S., 2013. Expression and treatment of pain-related behavioral depression. *Lab. Anim. (NY)* 42, 292–300.
- Negus, S.S., Vanderah, T.W., Brandt, M.R., Bilsky, E.J., Becerra, L., Borsook, D., 2006. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J. Pharmacol. Exp. Ther.* 319, 507–514.
- Nieto, F.R., Clark, A.K., Grist, J., Chapman, V., Malcangio, M., 2015. Calcitonin gene-related peptide-expressing sensory neurons and spinal microglial reactivity contribute to pain states in collagen-induced arthritis. *Arthritis Rheumatol.* 67, 1668–1677.
- Niiyama, Y., Kawamata, T., Yamamoto, J., Omote, K., Namiki, A., 2007. Bone cancer increases transient receptor potential vanilloid subfamily 1 expression within distinct subpopulations of dorsal root ganglion neurons. *Neuroscience* 148, 560–572.
- Overend, T.J., Wuori-Fearn, J.L., Kramer, J.F., MacDermid, J.C., 1999. Reliability of a patient-rated forearm evaluation questionnaire for patients with lateral epicondylitis. *J. Hand. Ther.* 12, 31–37.
- Pincus, T., Callahan, L.F., 1992. Rheumatology function tests: grip strength, walking time, button test and questionnaires document and predict longterm morbidity and mortality in rheumatoid arthritis. *J. Rheumatol.* 19, 1051–1057.
- Qu, Y.J., Zhang, X., Fan, Z.Z., Huai, J., Teng, Y.B., Zhang, Y., Yue, S.W., 2016. Effect of TRPV4-p38 MAPK pathway on neuropathic pain in rats with chronic compression of the dorsal root ganglion. *Biomed. Res. Int.* 2016, 6978923.
- Rice, D.A., McNair, P.J., Lewis, G.N., Dalbeth, N., 2015. The effects of joint aspiration and intra-articular corticosteroid injection on flexion reflex excitability, quadriceps strength and pain in individuals with knee synovitis: a prospective observational study. *Arthritis Res. Ther.* 17, 191.
- Romera, I., Montejo, A.L., Caballero, F., Caballero, L., Arbesu, J., Polavieja, P., Desai, D., Gilaberte, I., 2011. Functional impairment related to painful physical symptoms in patients with generalized anxiety disorder with or without comorbid major depressive disorder: post hoc analysis of a cross-sectional study. *BMC Psychiatry* 11, 69.
- Sánchez-Fernández, C., Nieto, F.R., González-Cano, R., Artacho-Cordón, A., Romero, L., Montilla-García, A., Zamanillo, D., Baeyens, J.M., Entrena, J.M.,

- Cobos, E.J., 2013. Potentiation of morphine-induced mechanical antinociception by sigma-1 receptor inhibition: role of peripheral sigma-1 receptors. *Neuropharmacology* 70, 348–358.
- Sarzi-Puttini, P., Vellucci, R., Zuccaro, S.M., Cherubino, P., Labianca, R., Fornasari, D., 2012. The appropriate treatment of chronic pain. *Clin. Drug Investig.* 32 (Suppl. 1), 21–33.
- Savilampi, J., Ahlstrand, R., Magnuson, A., Geijer, H., Wattwil, M., 2014. Aspiration induced by remifentanyl: a double-blind, randomized, crossover study in healthy volunteers. *Anesthesiology* 121, 52–58.
- Scanzello, C.R., Goldring, S.R., 2012. The role of synovitis in osteoarthritis pathogenesis. *Bone* 51, 249–257.
- Scott, D.L., Pugner, K., Kaarela, K., Doyle, D.V., Woolf, A., Holmes, J., Hieke, K., 2000. The links between joint damage and disability in rheumatoid arthritis. *Rheumatol. Oxf.* 39, 122–132.
- Shinoda, M., Ogino, A., Ozaki, N., Urano, H., Hironaka, K., Yasui, M., Sugiura, Y., 2008. Involvement of TRPV1 in nociceptive behavior in a rat model of cancer pain. *J. Pain* 9, 687–699.
- Sokolove, J., Lepus, C.M., 2013. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther. Adv. Musculoskelet. Dis.* 5, 77–94.
- St Pierre, M., Reeh, P.W., Zimmermann, K., 2009. Differential effects of TRPV channel block on polymodal activation of rat cutaneous nociceptors in vitro. *Exp. Brain Res.* 196, 31–44.
- Stubbs, D., Krebs, E., Bair, M., Damush, T., Wu, J., Sutherland, J., Kroenke, K., 2010. Sex differences in pain and pain-related disability among primary care patients with chronic musculoskeletal pain. *Pain Med.* 11, 232–239.
- Szallasi, A., Cortright, D.N., Blum, C.A., Eid, S.R., 2007. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nat. Rev. Drug Discov.* 6, 357–372.
- Turner, J.A., Ersek, M., Kemp, C., 2005. Self-efficacy for managing pain is associated with disability, depression, and pain coping among retirement community residents with chronic pain. *J. Pain* 6, 471–479.
- Unruh, A.M., 1996. Gender variations in clinical pain experience. *Pain* 65, 123–167.
- van Laarhoven, A.J., Kraaijaak, F.W., Wilder-Smith, O.H., van Riel, P.L., van de Kerkhof, P.C., Evers, A.W., 2013. Sensitivity to itch and pain in patients with psoriasis and rheumatoid arthritis. *Exp. Dermatol.* 22, 530–534.