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Non-invasive mechanical joint loading as an alternative model for osteoarthritic pain

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Abstract

Objective: Mechanisms responsible for osteoarthritic pain remain poorly understood and current analgesic therapies are often insufficient. We have characterized and pharmacologically tested the pain phenotype of a non-invasive mechanical joint loading (MJL) model of osteoarthritis thus providing an alternative murine model for osteoarthritic pain.

Methods: The right knees of male mice (12-week-old, C57BL/6) were loaded at 9N or 11N (40 cycles, three times/week for two weeks). Behavioural measurements of limb disuse, mechanical and thermal hypersensitivity were acquired before MJL and monitored for six weeks post-loading. The severity of articular cartilage lesions was determined post-mortem with the OARSI grading scheme. Furthermore, 9N-loaded mice were treated for four weeks with diclofenac (10mg/kg), gabapentin (100mg/kg) or anti-Nerve Growth Factor (3mg/kg).

Results: Mechanical hypersensitivity and weight-bearing worsened significantly in 9N- and 11N-loaded mice two weeks post-loading compared to baseline values and non-loaded controls. Maximum OA scores of ipsilateral knees confirmed increased cartilage lesions in 9N- (2.8±0.2) and 11N-loaded (5.3±0.3) mice compared to non-loaded controls (1.0±0.0). Gabapentin and diclofenac restored pain behaviours to baseline values after two weeks of daily treatment, with gabapentin being more effective than diclofenac. A single injection of anti-NGF alleviated nociception two days after treatment and remained effective for two weeks with a second dose inducing stronger and more prolonged analgesia.

Conclusion: Our results show that MJL induces OA lesions and a robust pain phenotype that can be reversed using analgesics known to alleviate OA pain in patients. This establishes the use of MJL as an alternative model for osteoarthritic pain.

Introduction

Osteoarthritis (OA) is a common degenerative joint disease associated with chronic, debilitating pain in the affected joints which significantly reduces the mobility and quality of life in patients [1]. Current therapies used to treat OA pain are often insufficient, with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) producing unwanted side effects which limit long-term use [2]. OA pathology and progression have been examined in detail, however, mechanisms contributing to osteoarthritic pain and the relationship between pain and OA pathology remain poorly understood.

To address this there is a need for a well characterized, non-invasive murine model of OA pain which exhibits both a robust, reproducible pain phenotype and histological evidence of OA pathology.

The two most commonly used models of OA in the preclinical field of osteoarthritic pain are the monosodium iodoacetate (MIA) model used to induce inflammatory OA [3] and surgical destabilization of the joint typically used to model post-traumatic OA [4, 5]. In the MIA model, a single intra-articular injection of MIA is placed in the knee joint which inhibits the glycolytic pathway causing chondrocytic cell death and an acute inflammation leading to cartilage erosion and joint disruption [6, 7]. The MIA injection causes immediate onset of mechanical hyperalgesia [8, 9], altered weight-bearing [10] and reduction in mobility [11] which are associated with the early, inflammatory phase (day 0-7). This is then followed by a more persistent allodynia typical for late phase OA (day 14-28). Pain-like behaviours increase in a dose-dependent manner, with late phase hypersensitivity typically observed at higher doses of MIA [12]. Surgical models, like the destabilization of the medial meniscus (DMM) [4] or the partial medial meniscectomy (PMM) [5, 13], are used predominantly in mice and rely on the surgical destabilization of the medial meniscus which typically leads to cartilage damage 4 to 8 weeks post-surgery [5, 14, 15]. Pain-like behaviours typically take longer to develop with mechanical hypersensitivity developing 4 weeks post-surgery, a decrease in spontaneous naturalistic behaviours seen 8 weeks post-surgery and an altered weight-bearing observed as late as 12 weeks post-surgery [13, 16, 17]. Mice undergoing sham surgery also show significant amounts of post-surgical pain [16, 17], with pain thresholds taking as long as 8 weeks to return to baseline levels [18]. A major drawback of both models is the invasiveness of the procedures which adds a layer of joint disruption that influences both joint damage and the resulting pain behaviours in affected as well as sham animals.

The non-invasive mechanical joint loading (MJL) model was initially used to investigate the osteogenic effect of mechanical loading on bone [19] and has recently been adapted to investigate the pathogeneses of OA [20]. The model induces OA through intermittent, repetitive loading of the tibia through the knee and ankle joints. Histological cartilage changes have been characterized in mice and show that single loading episodes induce lesions in the articular cartilage [20]. When loading episodes are repeated three times per week for two weeks these lesions spontaneously progress and worsen over a time frame of three weeks [20]. This model also shows changes in the subchondral bone [21] consistent with pathology seen in humans. This recent use of MJL as a model of OA means that the pain phenotype in this model has not yet been fully characterized.

The aim of this study was to characterize the pain phenotype of the murine MJL model of OA to determine if it can be used as a model of osteoarthritic pain. To this end we induced OA of different severity using two different load magnitudes and monitored hypersensitivity thresholds over time using an array of established behavioural assays developed in mice [22]. The presence of OA knee pathology was confirmed at the end of the study by quantifying cartilage damage. Furthermore, we investigated whether diclofenac, gabapentin or anti-Nerve Growth Factor monoclonal antibody (anti-NGF mAb) could alleviate the OA pain seen in this model. Diclofenac is an NSAID effective against inflammatory pain and the first-line treatment in the clinic for patients with OA pain [23] whilst gabapentin is an antiepileptic drug that is effective in complex neuropathic pain syndromes [24, 25]. Anti-NGF antibodies represent novel analgesics currently in clinical trials for OA pain [26-28]. *In vivo* studies show that anti-NGF treatment restores spontaneous day/night activity in mice with orthopaedic surgery-induced pain [29] and improves gait imbalance in both the MIA

model [30] and surgical model of OA [31]. Additionally, treatment with the soluble NGF receptor, TrkAD5, effectively restored the altered weight-bearing seen directly after DMM surgery (post-operative pain) as well as 16 days post-surgery (OA pain) [32]. Testing the efficacy of these drugs in alleviating pain is the first step in validating the MJL as an appropriate model for osteoarthritic pain.

Methods

Animals

Naïve male 10-week-old C57BL/6 mice (Charles River) were housed in groups of four in individually ventilated cages and fed a standard RM1 maintenance diet *ad libitum*. The environment was climate and light controlled; temperature 22°C, humidity 50%, lights on from 7AM-7PM. Animals were acclimatized for one week before start of procedures which were conducted during the light phase (8AM-6PM). All experiments were carried out in compliance with the Animals (Scientific Procedures) Act (1986) and approved by the UK Home Office license.

In vivo mechanical joint loading

Osteoarthritis was induced in mice by a two week loading regime [20] using an electronic materials testing machine (Bose 3100). Mice were 12 weeks old at the start of loading which was performed under general anaesthesia (3.5% isoflurane). The right tibia was positioned vertically between two custom-made loading cups which restrict the knee and ankle joints in deep flexion. Axial compressive loads were applied through the knee joint via the upper loading cup whilst a loading cell, attached to the lower cup, registered and monitored the applied loads. One loading cycle consists of 9.9 seconds holding time with a load magnitude of 2N (load needed to maintain knee position) after which a peak load of 9N or 11N was applied for 0.05 seconds with a rise and fall time of 0.025 seconds each. This 10 second trapezoidal wave loading cycle was repeated 40 times within one loading episode. During the loading regime this loading episode was repeated three times per week for two consecutive weeks. The load magnitudes of 9N or 11N were chosen to enable comparisons with previously published work on the loading model [19-21].

Experimental design

Pain phenotype after mechanical joint loading

Mice loaded at 9N or 11N (*n* = 8 / group) underwent behavioural measurements (see supplementary methods for overview) at baseline in the week before loading and were monitored weekly for six weeks post-loading. Changes in behaviour were compared to age and cage matched, non-loaded controls which were not subjected to any loading regime but instead underwent isoflurane anaesthesia for the same duration as loaded mice. No behavioural testing was performed during the two weeks of loading.

Pharmacological validation of mechanical joint loading

The MJL model was validated by testing the anti-nociceptive effect of diclofenac, gabapentin and an anti-NGF mAb on 9N-loaded mice. In total six groups (*n* = 8/group) were tested; three experimental groups in which one dose of each drug was tested and three control groups; non-loaded saline-treated, loaded saline-treated and loaded inactive control antibody treated. Analgesic treatment was administered from two to six weeks post-loading. Animals receiving diclofenac (10 mg/kg, p.o. [33], Sigma-Aldrich), gabapentin (100 mg/kg, p.o. [33], Sigma-Aldrich) or saline (0.9% NaCl, p.o., Sigma-Aldrich) were treated daily via gavage without anaesthesia. The volume administered was calculated according to the weight of the animal (<500µL). Anti-NGF mAb treatment (3 mg/kg, i.p., MEDI578 Batch: SP10-291, generously gifted by AstraZeneca, MedImmune) was administered at weeks two and four post-loading. Loaded controls received the inactive antibody (3 mg/kg, i.p., NIP228 IgG4P control Batch: SP-15-302, AstraZeneca, MedImmune) at same time-points.

Pain thresholds were measured at baseline and continued after loading on a weekly basis in mice receiving saline, diclofenac or gabapentin. Behavioural testing started one hour after treatment. Animals receiving anti-NGF or inactive antibody were tested 4, 24 and 48 hours and then every two days following treatment.

Histological analysis of joints and OA grading

Six weeks post-loading, mice were euthanized by CO_2 overdose followed by cervical dislocation. Hind limbs were removed and fixed in 4% neutral buffered formalin for 48 hours. Knees were then decalcified (Immunocal, Quartett) for 5 days and processed for paraffin embedding. Once embedded, 6µm coronal sequential sections were acquired of the entire joint, of which a quarter was stained with toluidine blue (0.1% in 0.1M acetate buffer, pH 5.6). OA severity was scored for each stained section using a grading system [34] ranging from 0-6. Briefly, grade 0 corresponds to normal surface articular cartilage; grade 0.5, a loss of toluidine blue staining; grade 1, lesions in the superficial zone of the articular cartilage; grade 2, lesions down to the intermediate zone; grade 3,

lesions down to the tidemark with possible loss of articular cartilage up to 20% of the surface of the condyle; grade 4, loss of 20% to 50% articular cartilage; grade 5 loss of 50-80% of articular cartilage; and finally, grade 6, with above 80% articular cartilage loss and exposure of subchondral bone. For each knee the maximum OA score, as determined by the lesion with the highest severity, and a summed OA score is reported. OA severity is classified as either low (grade 0-2), mild (grade 3-4) or severe (grade 5-6).

Statistical analysis

Data were analysed using GraphPad Prism (7.04). Results are presented as mean ± SEM. Mice were assigned conditions in a pseudo-random order, ensuring comparable behavioural baseline values and allocating different conditions within the home cage. Two mice in the diclofenac treatment group were excluded from analysis due to adverse gastro-intestinal effects. After checking for normal distribution, multiple groups were compared using parametric two-way ANOVA followed by a Bonferroni post hoc test. Values of p less than 0.05 were considered as statistically significant.

Results

MJL at both 9N and 11N induces chronic mechanical hypersensitivity combined with altered weight-bearing and reduced mobility.

MJL with a load of either 9N or 11N induces a mechanical pain phenotype which is established two weeks post-loading and progressively worsens until six weeks post-loading. From two to six weeks post-loading, 9N- (figure 1A) and 11N-loaded (figure 1C) mice show a significant and persisting reduction in mechanical sensitivity compared to both baseline values and non-loaded controls (for 9N- and 11N-loaded mice; $p<0.001$). Mice loaded at 9N show a reduction from baseline (0.513g±0.06g) to two weeks post loading (0.207g±0.04g) with thresholds progressively lowering further till 6 weeks post-loading (0.131g±0.03g). 11N-loaded mice show a similar trend with baseline mechanical threshold (0.505g±0.08g) dropping 2 weeks post-loading (0.165g±0.03g) and stabilizing up to 6 weeks post-loading (0.108g±0.03g). Notably, there is also a reduction in the mechanical sensitivity thresholds of the contralateral paw, although this develops at a later stage and is not as pronounced as in the ipsilateral paw (figure 1B and D, respectively).

The development of mechanical hypersensitivity was accompanied by altered weight-bearing and reduction in mobility. 9N-loaded mice progressively reduced the percentage of weight borne on the ipsilateral hind limb from baseline (49.94%±0.6%) to 4 weeks post-loading (44.15%±1.4%) which is significantly different to weight-bearing values in non-loaded controls ($p=0.0024$, figure 2A). Mice loaded at 11N also showed a decrease in ipsilateral weight-bearing over

time significantly different to non-loaded controls (p=0.0343, figure 2C); with values decreasing from baseline (49.41%±0.3%) to 2 weeks post-loading (41.03%±2.5%) but then returning 4 weeks post-loading (47.67%±1.5%) to finally decrease again at 6 weeks post-loading (44.23%±2.1%.).

Motor ability was slightly reduced in the 9N-loaded mice which showed a decline in time spent on the rotarod compared to non-loaded control mice (p=0.0238). This reached significance at six weeks post-loading (figure 2B). 11N-loaded mice exhibit a similar decline in time spent on the rotarod which is significance 5 weeks post-loading (p=0.0071, figure 2D).

Thermal sensitivity as determined by the hot plate (50°C and 55°C), cold plate (0°C), Hargreaves and cold plantar assay measurements showed no difference in thresholds between loaded and non-loaded animals (results not shown). The non-loaded control group did not show changes over time in any of the pain measurements.

MJL at both 9N and 11N induces articular cartilage lesions.

Histological analysis of joints revealed that loading at both 9N and 11N induced OA lesions in ipsilateral and contralateral knees, with higher maximum (figure 3A) and summed (figure 3B) severity scores compared to the non-loaded controls. Maximum ipsilateral articular cartilage lesions were higher in 11N-loaded mice (5.3±0.3) compared to 9N-loaded mice (2.8±0.2, p<0.001, figures 3C-H). The development of lesions seen in 9N-loaded mice at one, three and six weeks post-loading is shown in the supplementary data. Additionally, the contralateral knees showed mild OA lesions in both 9N (1.8±0.2) and 11N-loaded (2.1±0.5) mice. The extreme OA pathology seen in 11N-loaded mice compared to that seen in the 9N-loaded mice led to a 9N loading regime to be used in the pharmacological study.

Treatment with diclofenac, gabapentin and anti-NGF mAb at two weeks post-loading relieves the mechanical hypersensitivity and improves the weight distribution without affecting motor ability.

For the pharmacological validation of the MJL model all animals were loaded at 9N. The non-loaded, saline-treated animals showed no change in nociceptive thresholds over time whilst the loaded saline-treated group exhibited mechanical hypersensitivity and altered weight-bearing from 2 weeks post-loading as previously shown (figure 4). Both gabapentin and diclofenac relieved the mechanical hypersensitivity (figure 4A) and the altered weight-bearing (figure 4B) after two weeks of treatment, with gabapentin being more effective than diclofenac. Two weeks of gabapentin treatment increased the mechanical threshold (1.234g±0.11g) compared to that before treatment (0.148g±0.03g), making mechanical sensitivity significantly higher compared to loaded, saline-treated mice, (p<0.001, figure 4A). In comparison, diclofenac increased mechanical thresholds (0.083g±0.02g) after two weeks of treatment (0.472g±0.09g, p=0.0057 when compared to loaded, saline-treated mice, figure 4A). Both gabapentin and diclofenac effectively reversed the altered

weight-bearing after two weeks of treatment; gabapentin (46.62%±3.0%) and diclofenac (47.23%±2.1%) compared to loaded, saline-treated mice (39.91%±2.1%, figure 4B).

The first injection of anti-NGF antibody effectively alleviated loading-induced pain behaviours (figure 5), with the second injection showing a stronger and more prolonged analgesic effect. Two days after the first treatment, the anti-NGF mAb significantly alleviated mechanical hypersensitivity (0.360g±0.08g) compared to inactive antibody-treated animals (0.117g±0.02g, *p*=0.028). This lasted for four days after which the effect dwindled. The second treatment with anti-NGF mAb was also effective two days post-injection inducing a cumulative effect with mechanical sensitivity returning to and exceeding baseline values (0.820g±0.10g), compared to animals treated with inactive antibody (0.072g±0.01g, *p*<0.001). Effectiveness of the second anti-NGF mAb treatment lasted up to 13 days post-injection (figure 5A). Weight-bearing results show a similar pattern with anti-NGF mAb treatment restoring weight-distribution one week post-injection but losing effectiveness two weeks later (figure 5B). A week after the second anti-NGF mAb treatment, animals showed significantly improved weight-bearing (51.44%±1.9%) compared to animals treated with inactive antibody (38.76%±1.0%, *p*<0.001, figure 5B).

All treatment groups showed a similar decline mobility, as measured by the rotarod, compared to loaded controls (saline-treated; figure 6A or inactive antibody treated; figure 6B), except for diclofenac-treated animals which did not show a decrease in time spent on the rotarod (figure 6A). Furthermore, exploratory behaviour was the same in all groups (figure 6C) and none of the treatments influenced weight gain (data not shown).

Discussion

In this study, we demonstrate that mechanical joint loading (MJL) is an appropriate model to specifically study mechanically-induced osteoarthritic pain. We have characterized the symptomatic aspects of mechanically-induced osteoarthritis by measuring the development of nociceptive behaviour alongside a histopathological presence of OA. Furthermore, the first step was taken in validating the MJL model by showing alleviation of nociceptive behaviour when treated with different classes of analgesics.

Mechanical loading of joints is known to induce alterations in articular cartilage [35] which, in cases of repetitive or excessive loading, can lead to osteoarthritis [36]. The MJL model has been developed to explore the mechanisms responsible for mechanically-induced osteoarthritis [20]. It mimics structural changes typically seen in human OA such as spontaneously progressing articular cartilage lesions, subchondral bone changes and osteophyte formation [20, 21]. The non-invasive nature of this model has an added value of enabling examination of whole joint pathology in an intact knee. This avoids complications typical for surgical interventions, like post-surgery pain and infection risk, thus increasing animal welfare and reducing variance in behavioural measurements.

This is the first measurement of pain behaviours in this model. MJL at both 9N and 11N induces mechanical hypersensitivity accompanied by altered weight-bearing and reduced mobility, without affecting thermal sensitivity. The development of pain-like behaviours is comparable for both loading intensities, with ipsilateral mechanical hypersensitivity and altered weight-bearing developing from two weeks post-loading and contralateral mechanical hypersensitivity, as well as reduced mobility, developing 4-5 weeks post-loading. This pain phenotype is similar to the pain observed in OA patients that initially presents with hypersensitivity of the affected joint and pain during weight-bearing. Frequency, duration and severity of pain worsen as OA progresses and peripheral as well as central neurological mechanisms are recruited, which leads to centralized allodynia common for late stage OA [37]. Consequently, the contralateral mechanical hypersensitivity observed after MJL could be due to altered gait [21] where mice relieve ipsilateral hypersensitivity by compensating with their contralateral limb or, alternatively, it could indicate a centralized hypersensitivity. No significant changes in behavioural measurements were observed in the first week after loading which suggesting that progressive mechano-adaptive changes over time, rather than the initial insult of mechanical loading, are responsible for the nociceptive behaviour. Further studies show that the initial cartilage lesions induced by MJL at 9N worsen over time, matching the progressive nature in the development of nociceptive behaviour (see supplementary data). Taken together these results suggest that MJL induces a nociceptive phenotype typical for progressive, mechanically induced OA.

This nociceptive phenotype seen after MJL, is more comparable with surgical models of OA than with the MIA model. The MIA model typically shows a stark increase in mechanical hypersensitivity and altered weight-bearing immediately after injection which persists up to 28 days post injection [8, 12, 38] whereas the MJL model does not show this immediate nociceptive response typical of the inflammatory form of OA seen in the MIA model. Furthermore, MIA injections in mice do not induce a reduction in motor ability [12, 38], or any contralateral nociceptive behaiour. In the DMM model mechanical hypersensitivity develops 2-4 weeks post-surgery and lasts up to 16 weeks [18], with altered weight-bearing taking up to 12 weeks to develop [16] and no change in locomotion or thermal sensitivity [39]. Although the onset of nociceptive behaviours appears earlier in the MJL model, the delay in behavioural responses seen in both MJL and DMM models is common for a progressive form of OA. Additionally, DMM induces contralateral nociceptive behaviours [39] comparable to those seen in the MJL model, indicating compensatory behaviour or central hypersensitization. In contrast, the MJL model does not show any post-surgical pain or hypersensitivity in sham animals that is typical for surgical models [5, 17]. Rather than relying on inflammatory damage of the joint as shown in the MIA model, both the MJL and the DMM models rely on a mechanical disruption and joint destabilization similar to that seen in human OA where excessive use or trauma leads to progressive joint damage.

A general drawback of this model is that there is no sham procedure which can control for, or rule out, off target damage induced by the loading procedure. The non-loaded controls used do not get loaded but are subjected to the anaesthesia procedure and, consequently, function as behavioural controls rather than controls for knee pathologies not related to mechanically-induced OA. Mice loaded statically at 2N (data not shown) show mild ipsilateral mechanical hypersensitivity which is neither consistent nor progressive. Additionally, these mice exhibit mild ipsilateral lesions in the articular cartilage. This makes the 2N-loaded mice inappropriate as controls for osteoarthritic pain.

At six weeks post-loading, both 9N- and 11N-loaded mice show lesions in the articular cartilage of ipsilateral and contralateral knees, confirming that MJL induces an OA-like histopathological phenotype. Analysis of articular cartilage lesions at one, three and six weeks post-loading at 9N (supplementary data) show that lesions in this study are comparable to those described by Poulet *et al*. [20] 3 weeks post-loading in mice with the same loading regime. Additionally, these results confirm the spontaneous exacerbation of lesions at 3 weeks post-loading compared to lesions seen directly after loading. The time frame in which these lesions progress and worsen corresponds to the development of nociceptive behaviours in this model, suggesting that the progressive degradation of the knee induces this behaviour. Furthermore, both 9N- and 11N-loaded mice showed mild contralateral damage which could explain the development of contralateral mechanical hypersensitivity seen in these animals.

Notably, 11N-loaded mice had extensive ipsilateral damage, with lesions consistently reaching maximum scores, whilst 9N-loaded mice showed milder OA histopathology without heightened nociceptive behaviour. This implies that, although cartilage damage is an important indicator of OA, it does not necessarily relate to the severity of pain. Pro-osteogenic changes in the tibia, for which this model was originally developed, are typically only seen at loading magnitudes of 13N or higher [19]. With the loading regimes of 9N or 11N no such osteogenic effects were observed (data not shown), indicating that bone remodelling of the tibia does not contribute to the MJL-induced development of nociceptive behaviour. Knee OA is a whole joint disease and, in patients with OA, moderate correlations between pain severity and MRI or radiograph read-outs of tissue damage have been shown for a variety of knee tissues including joint space narrowing [40], subchondral bone changes, synovitis and meniscal tears [41]. Additional experiments will be needed to study the effect of MJL on other joint tissues and identify their role in the development of nociceptive behaviour. The lack of difference in pain profile seen between the 9N- and 11N-loaded mice could reflect the modest sensitivity of pain read-outs used, all of which are measurements for referred pain. However, results from this study clearly show that 11N-loaded mice develop the maximum possible knee damage, thus reaching a ceiling effect in both OA severity score and pain phenotype. The severe knee pathology seen in these mice could indicate that MJL at 11N induces damage which is not restricted to the cartilage but also affects other joint tissues. Combined with the observation that the 9N-loaded mice develop a milder form of OA but still show a robust pain phenotype it was concluded that loading regime at 9N was more appropriate for follow-up pharmacology studies.

Diclofenac, gabapentin and anti-NGF mAb, all analgesics used treat OA pain in patients, were effective in alleviating MJL-induced nociceptive behaviour. Additionally, these treatments had no effect on the exploratory behaviour or weight of the mice demonstrating that animal welfare was not compromised. We also showed that none of these treatments compromised mobility, suggesting that the restoration of behavioural responses to baseline values was due to their analgesic effects rather than possible sedative side effects or motor impairment.

In the first two weeks of treatment, gabapentin was more effective in alleviating mechanical hypersensitivity and restoring weight-bearing than diclofenac. This is particularly striking considering that diclofenac, which is typically effective in treating inflammatory pain, is the first line treatment for OA whilst gabapentin is more commonly used to treat neuropathic pain. Despite the preferential effectiveness against neuropathic pain, gabapentin has been shown to be effective in treating

nociception in both MIA [42] and surgical models [43, 44] of OA. The efficacy of gabapentin in several OA pain models suggests that OA pain could in part be of neuropathic origin. In fact, in the PMM model of OA, diclofenac was only effective in treating nociception in the initial inflammatory phase but not at a later stage, whereas gabapentin alleviated the mechanical hypersensitivity seen in the chronic phase of OA-induced nociception [5]. Taken together, this suggests that although inflammation and the resulting pain do likely play a role in OA pathology, OA is a complex pain syndrome with a significant neuropathic component.

The anti-NGF antibody showed a prolonged and significant reduction in nociceptive hypersensitivity with repeated treatment increasing the magnitude and duration of its effectiveness. There is a lot of evidence supporting a role for NGF in osteoarthritic pain [45]. Chondrocytes produce NGF in response to degeneration, NGF levels are elevated in the synovial fluid of patients with OA and in clinical trials anti-NGF mAb treatment has provided significant pain relief in OA patients [46]. Furthermore, in the MIA and medial meniscal transection murine models of OA, intra-articular injections of NGF increased nociceptive behavioural responses in both experimental and healthy control animals suggesting that NGF plays a role in the severity of OA pain [47]. The prolonged effectiveness of anti-NGF mAb treatment in the MJL model is similar to the MIA model where anti-NGF effectively restored altered gait for up to 35 days post treatment [30, 48].

Historically, several murine models of OA have been useful in unravelling mechanisms of pathogenesis of this condition. The ease of genetic modification, the relative low costs and reduced time needed for disease progression make mice widely used in both OA and pain research. Here we present an alternative model that closely mimics an OA phenotype typical for mechanically-induced OA. Our results show that the non-invasive mechanical joint loading model induces both OA lesions and a reproducible pain phenotype which can be reversed using known analgesics for OA pain, thus suggesting its use as an alternative model to study osteoarthritic pain.

References

1.   Martel-Pelletier, J., et al., *Osteoarthritis.* Nat Rev Dis Primers, 2016. **2**: p. 16072.

2.   Hochberg, M.C., et al., *American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee.* Arthritis Care Res (Hoboken), 2012. **64**(4): p. 465-74.

3.   Pitcher, T., J. Sousa-Valente, and M. Malcangio, *The Monoiodoacetate Model of Osteoarthritis Pain in the Mouse.* J Vis Exp, 2016(111).

4.   Glasson, S.S., T.J. Blanchet, and E.A. Morris, *The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse.* Osteoarthritis Cartilage, 2007. **15**(9): p. 1061-9.

5.   Knights, C.B., C. Gentry, and S. Bevan, *Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice.* Pain, 2012. **153**(2): p. 281-92.

6.   Guzman, R.E., et al., *Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis.* Toxicol Pathol, 2003. **31**(6): p. 619-24.

7.   van der Kraan, P.M., et al., *Degenerative knee joint lesions in mice after a single intra-articular collagenase injection. A new model of osteoarthritis.* J Exp Pathol (Oxford), 1990. **71**(1): p. 19-31.

8.   Ogbonna, A.C., A.K. Clark, and M. Malcangio, *Development of monosodium acetate-induced osteoarthritis and inflammatory pain in ageing mice.* Age (Dordr), 2015. **37**(3): p. 9792.

9.   Fernihough, J., et al., *Pain related behaviour in two models of osteoarthritis in the rat knee.* Pain, 2004. **112**(1-2): p. 83-93.

10.   Bove, S.E., et al., *Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis.* Osteoarthritis Cartilage, 2003. **11**(11): p. 821-30.

11.   Guingamp, C., et al., *Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology, and biochemistry.* Arthritis Rheum, 1997. **40**(9): p. 1670-9.

12.   Ogbonna, A.C., et al., *Pain-like behaviour and spinal changes in the monosodium iodoacetate model of osteoarthritis in C57Bl/6 mice.* Eur J Pain, 2013. **17**(4): p. 514-26.

13.   Driscoll, C., et al., *Nociceptive Sensitizers Are Regulated in Damaged Joint Tissues, Including Articular Cartilage, When Osteoarthritic Mice Display Pain Behavior.* Arthritis Rheumatol, 2016. **68**(4): p. 857-67.

14.   Loeser, R.F., et al., *Disease progression and phasic changes in gene expression in a mouse model of osteoarthritis.* PLoS One, 2013. **8**(1): p. e54633.

15.   Kim, B.J., et al., *Establishment of a reliable and reproducible murine osteoarthritis model.* Osteoarthritis Cartilage, 2013. **21**(12): p. 2013-20.

16.   Inglis, J.J., et al., *Regulation of pain sensitivity in experimental osteoarthritis by the endogenous peripheral opioid system.* Arthritis Rheum, 2008. **58**(10): p. 3110-9.

17.   Miller, R.E., et al., *CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis.* Proc Natl Acad Sci U S A, 2012. **109**(50): p. 20602-7.

18.   Miller, R.E., et al., *Chemogenetic Inhibition of Pain Neurons in a Mouse Model of Osteoarthritis.* Arthritis Rheumatol, 2017. **69**(7): p. 1429-1439.

19.   De Souza, R.L., et al., *Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element.* Bone, 2005. **37**(6): p. 810-8.

20.   Poulet, B., et al., *Characterizing a novel and adjustable noninvasive murine joint loading model.* Arthritis Rheum, 2011. **63**(1): p. 137-47.

21.   Poulet, B., et al., *Intermittent applied mechanical loading induces subchondral bone thickening that may be intensified locally by contiguous articular cartilage lesions.* Osteoarthritis Cartilage, 2015. **23**(6): p. 940-8.

22.   Minett, M.S., K. Quick, and J.N. Wood, *Behavioral Measures of Pain Thresholds.* Curr Protoc Mouse Biol, 2011. **1**(3): p. 383-412.

23.   Atzeni, F., I.F. Masala, and P. Sarzi-Puttini, *A Review of Chronic Musculoskeletal Pain: Central and Peripheral Effects of Diclofenac.* Pain Ther, 2018.

24.   Enggaard, T.P., et al., *The effects of gabapentin in human experimental pain models.* Scand J Pain, 2017. **1**(3): p. 143-148.

25.   Murnion, B.P., *Neuropathic pain: current definition and review of drug treatment.* Aust Prescr, 2018. **41**(3): p. 60-63.

26.   Lane, N.E., et al., *Tanezumab for the treatment of pain from osteoarthritis of the knee.* N Engl J Med, 2010. **363**(16): p. 1521-31.

27.   Schnitzer, T.J., et al., *Long-term open-label study of tanezumab for moderate to severe osteoarthritic knee pain.* Osteoarthritis Cartilage, 2011. **19**(6): p. 639-46.

28.   Brown, M.T., et al., *Tanezumab reduces osteoarthritic knee pain: results of a randomized, double-blind, placebo-controlled phase III trial.* J Pain, 2012. **13**(8): p. 790-8.

29.   Majuta, L.A., et al., *Anti-nerve growth factor therapy increases spontaneous day/night activity in mice with orthopedic surgery-induced pain.* Pain, 2017. **158**(4): p. 605-617.

30.   Miyagi, M., et al., *Efficacy of nerve growth factor antibody in a knee osteoarthritis pain model in mice.* BMC Musculoskelet Disord, 2017. **18**(1): p. 428.

31.   LaBranche, T.P., et al., *Nerve growth factor inhibition with tanezumab influences weight-bearing and subsequent cartilage damage in the rat medial meniscal tear model.* Ann Rheum Dis, 2017. **76**(1): p. 295-302.

32.   McNamee, K.E., et al., *Treatment of murine osteoarthritis with TrkAd5 reveals a pivotal role for nerve growth factor in non-inflammatory joint pain.* Pain, 2010. **149**(2): p. 386-92.

33.   Suokas, A.K., et al., *Design, study quality and evidence of analgesic efficacy in studies of drugs in models of OA pain: a systematic review and a meta-analysis.* Osteoarthritis Cartilage, 2014. **22**(9): p. 1207-23.

34.   Glasson, S.S., et al., *The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse.* Osteoarthritis Cartilage, 2010. **18 Suppl 3**: p. S17-23.

35.   Kiviranta, I., et al., *Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs.* J Orthop Res, 1988. **6**(2): p. 188-95.

36.   Kujala, U.M., et al., *Knee osteoarthritis in former runners, soccer players, weight lifters, and shooters.* Arthritis Rheum, 1995. **38**(4): p. 539-46.

37.   Fingleton, C., et al., *Pain sensitization in people with knee osteoarthritis: a systematic review and meta-analysis.* Osteoarthritis Cartilage, 2015. **23**(7): p. 1043-56.

38.   Harvey, V.L. and A.H. Dickenson, *Behavioural and electrophysiological characterisation of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice.* Mol Pain, 2009. **5**: p. 18.

39.   Malfait, A.M., et al., *ADAMTS-5 deficient mice do not develop mechanical allodynia associated with osteoarthritis following medial meniscal destabilization.* Osteoarthritis Cartilage, 2010. **18**(4): p. 572-80.

40.   Neogi, T., et al., *Association between radiographic features of knee osteoarthritis and pain: results from two cohort studies.* BMJ, 2009. **339**: p. b2844.

41.   Torres, L., et al., *The relationship between specific tissue lesions and pain severity in persons with knee osteoarthritis.* Osteoarthritis Cartilage, 2006. **14**(10): p. 1033-40.

42.   Vonsy, J.L., J. Ghandehari, and A.H. Dickenson, *Differential analgesic effects of morphine and gabapentin on behavioural measures of pain and disability in a model of osteoarthritis pain in rats.* Eur J Pain, 2009. **13**(8): p. 786-93.

43.   Kamath, R.V., et al., *Development and validation of mechanical allodynia as a pain readout in a preclinical model of osteoarthritis.* Osteoarthritis and Cartilage, 2012. **20**: p. S62.

44.   Bove, S.E., et al., *Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia.* Osteoarthritis Cartilage, 2006. **14**(10): p. 1041-8.

45.   Shang, X., Z. Wang, and H. Tao, *Mechanism and therapeutic effectiveness of nerve growth factor in osteoarthritis pain.* Ther Clin Risk Manag, 2017. **13**: p. 951-956.

46.   Walicke, P.A., et al., *First-in-human randomized clinical trials of the safety and efficacy of tanezumab for treatment of chronic knee osteoarthritis pain or acute bunionectomy pain.* Pain Rep, 2018. **3**(3): p. e653.

47.   Ashraf, S., et al., *Augmented pain behavioural responses to intra-articular injection of nerve growth factor in two animal models of osteoarthritis.* Ann Rheum Dis, 2014. **73**(9): p. 1710-8.

48.   Ishikawa, G., et al., *Efficacy of drugs with different mechanisms of action in relieving spontaneous pain at rest and during movement in a rat model of osteoarthritis.* Eur J Pharmacol, 2014. **738**: p. 111-7.

Figure Legends

Figure 1: Development of mechanical hypersensitivity after MJL.

The right knees of mice were loaded three times per week for two weeks at 9N (red line, *n* = 8) or 11N (green line, *n* = 8) to induce OA. Development of mechanical hypersensitivity was measured using von Frey filaments (50% paw withdrawal threshold (PWT) in grams) in the ipsilateral (**A**, 9N and **C**, 11N) and contralateral paws (**B**, 9N and **D**, 11N). The values were compared to a non-loaded isoflurane control (black dotted line, *n* = 8). Significant changes between non-loaded and loaded animals are indicated with a # ($p < 0.05$), ## ($p < 0.01$) or ### ($p < 0.001$) whilst significant changes within groups over time (compared to baseline value) are indicated with a * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$). Values given as the mean ± SEM

Figure 2: Altered weight-bearing and reduced mobility after MJL.

The right knees of mice were loaded three times per week for two weeks at 9N (red line, n = 8) or 11N (green line, n = 8) to induce OA. Altered weight-bearing (weight placed on ipsilateral paw as a percentage of total weight placed on both legs) was measured using the incapacitance test (**A**, 9N and **C**, 11N). Motor ability was measured using the rotarod (duration mice were able to remain on the rotarod; **B**, 9N and **D**, 11N). The values were compared to a non-loaded isoflurane control (black dotted line, n = 8). Significant changes between non-loaded and loaded animals are indicated with a # (p < 0.05), ## (p < 0.01) or ### (p < 0.001) whilst significant changes within groups over time (compared to baseline value) are indicated with a * (p < 0.05) or ** (p < 0.01). Values given as mean ± SEM

Figure 3: Severity of OA lesions after MJL at 9N and 11N.

Ipsilateral and contralateral knees of non-loaded and loaded mice at 9N and 11N were collected post mortem at 6 weeks post-loading and OA severity was scored (scoring system from 0-6, OA severity is classified as either low (grade 0-2), mild (grade 3-4) or severe (grade 5-6)). Maximum OA scores (**A**) and summed OA scores (**B**) are given for non-loaded (black circles, n = 6), 9N-loaded mice (red squares, n = 6) and 11N- loaded mice (green triangles, n = 6). Significant differences in the severity of OA lesions indicated with a # (p < 0.05), ## (p < 0.01) or ### (p < 0.001). Values given as mean ± SEM. Examples of typical knee histology of the ipsilateral knee are shown for non-loaded (**C** and **D**), 9N-loaded (**E** and **F**) and 11N-loaded mice (**G** and **H**), with panels B, D and F showing whole knee joint and panels C, E and G showing the medial compartment at 10x magnification. Arrows indicate typical cartilage damage seen for each condition.

Figure 4: Effect of diclofenac and gabapentin treatment on post-loading mechanical hypersensitivity and altered weight-bearing.

Daily analgesic treatments were started two weeks post-loading indicated with grey, dotted line. Mechanical hypersensitivity (**A**, 50% paw withdrawal threshold (PWT) in grams) and weight-bearing (**B**, weight placed on ipsilateral paw as a percentage of total weight placed on both legs) was monitored on a weekly basis for animals receiving saline (non-loaded controls; black dotted line, and 9N-loaded controls; red line, n = 8), diclofenac (10 mg/kg p.o., green line, n = 6) or gabapentin (100 mg/kg p.o., blue line, n = 8) treatment. Significant changes between treated and saline-treated 9N-loaded groups are indicated with a $ (p < 0.05), $$ (p < 0.05) or $$$ (p < 0.05) whilst significant changes within groups over time (compared to baseline value) are indicated with a * (p <0 .05), or *** (p < 0.001) in corresponding colours. Values given as the mean ± SEM
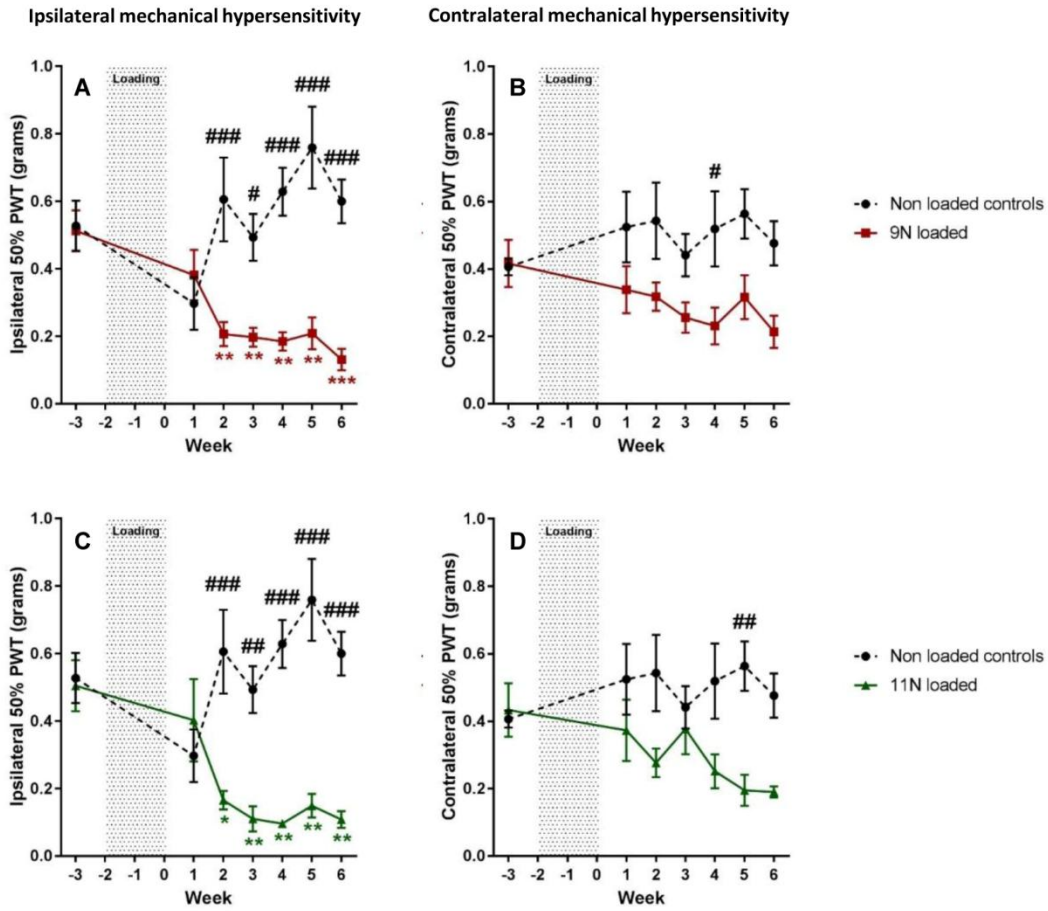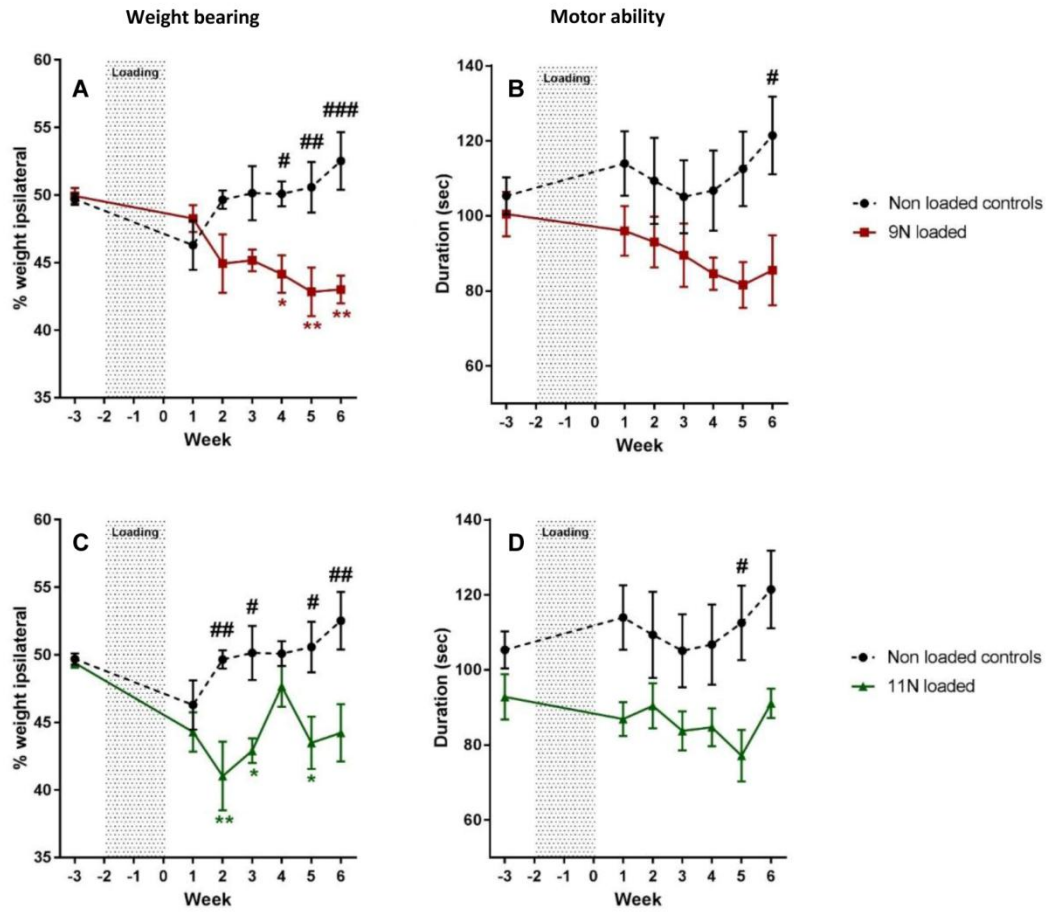
Figure 5: Effect of anti-NGF mAb treatment on post-loading mechanical hypersensitivity and altered weight-bearing.

Animals received anti-NGF mAb treatment two and four weeks post-loading, indicated in both cases with grey dotted lines. Mechanical hypersensitivity was initially monitored on a weekly basis and then on a more frequent basis after animals started receiving anti-NGF (MEDI578, 3 mg/kg i.p., black line, n = 8) or inactive antibody (NIP228, 3 mg/kg i.p., red dotted line, n = 8) treatment. Panel A; 50% paw withdrawal threshold (PWT) in grams and behavioural days indicated in blue as number of days post-treatment. Weight-bearing was monitored on a weekly basis (**B**; weight placed on ipsilateral paw as a percentage of total weight placed on both legs). Significant changes between treated groups and saline treated 9N-loaded groups are indicated with a $ ($p < 0.05$) or $$$ ($p < 0.05$) whilst significant changes within groups over time (compared to baseline value) are indicated with a * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$) in corresponding colours. Values given as the mean ± SEM
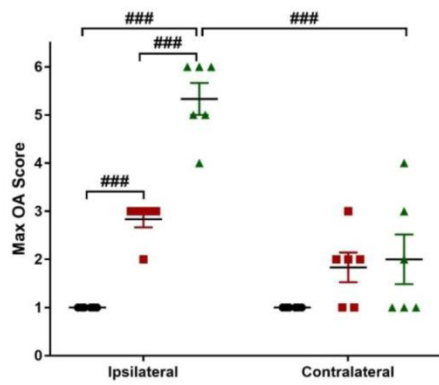
Figure 6: Effect of analgesic treatment on post-loading motor ability and natural exploratory behaviour.

Motor activity was assessed by the rotarod (duration mice were able to remain on rotaod in seconds). Panel A shows controls animals receiving saline (non-loaded controls; black dotted line, n = 8 and 9N-loaded controls; red line, n = 8), diclofenac (10 mg/kg p.o., green line, n = 6) or gabapentin (100 mg/kg p.o., blue line, n = 8) treatment and panel B shows animals receiving anti-NGF (MEDI578, 3 mg/kg i.p., black line, n = 8) or inactive antibody (NIP228, 3 mg/kg i.p., red dotted line, n = 8) treatment. Significant changes within groups compared to baseline values are indicated with a * ($p < 0.05$) or *** ($p < 0.001$) in corresponding colours. Natural exploratory behaviour as measured with the open field was also unaffected by treatment as shown in panel C (of crossing in the open field over 5 minutes). Values given as mean ± SEM

Ipsilateral mechanical hypersensitivity

Contralateral mechanical hypersensitivity

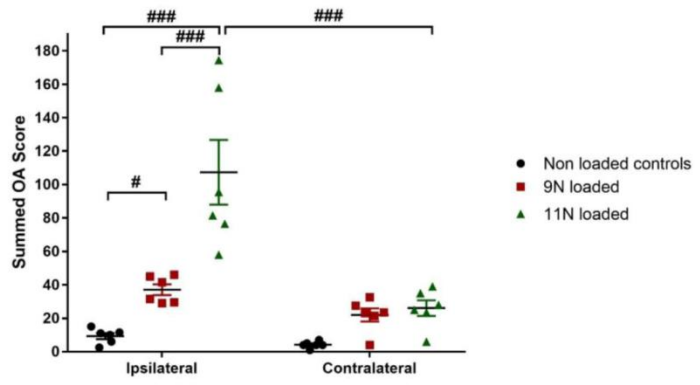**Weight bearing**

Motor ability
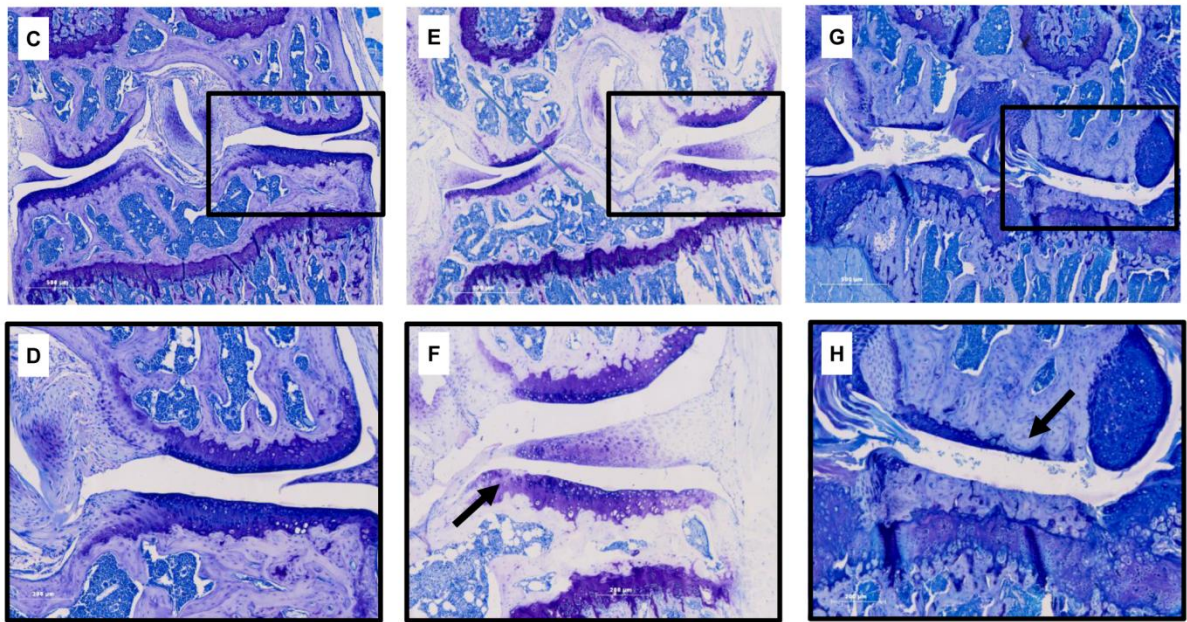
A: Maximum severity of cartilage lesions

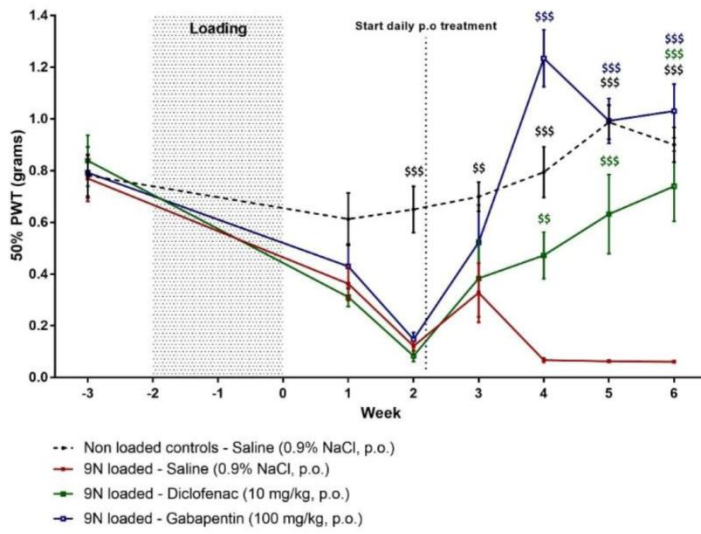B: Summed severity of cartilage lesions

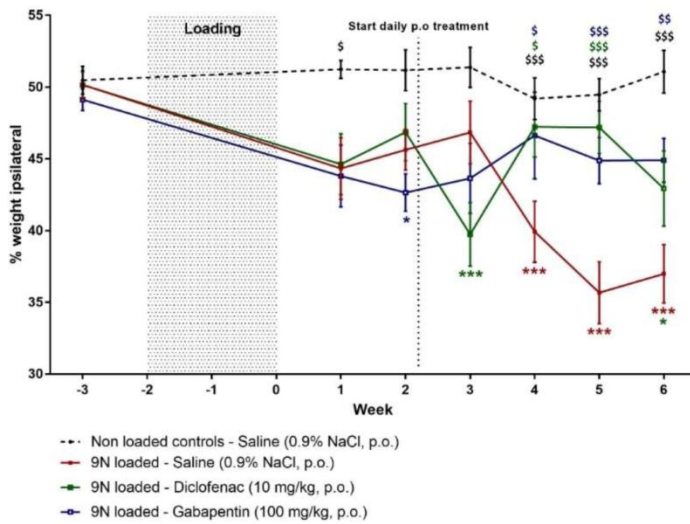Non loaded controls

9N loaded

11N loaded

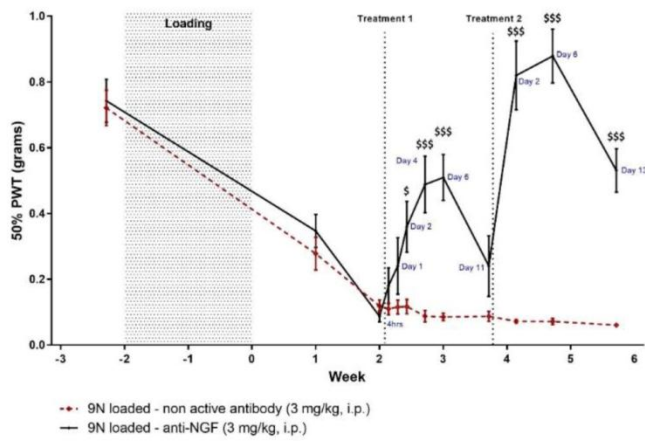## A. Ipsilateral mechanical hypersensitivity : diclofenac/gabapentin treatment

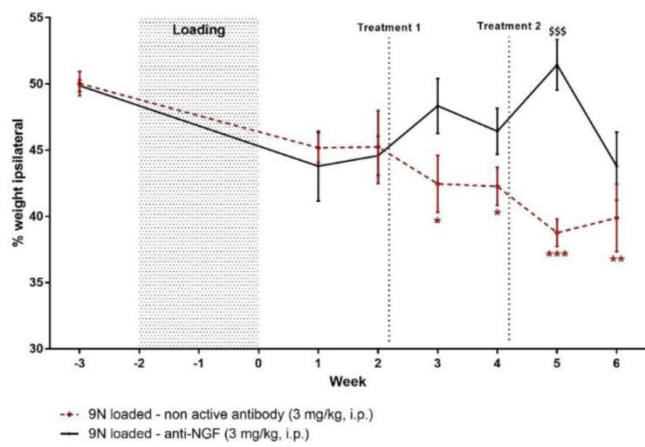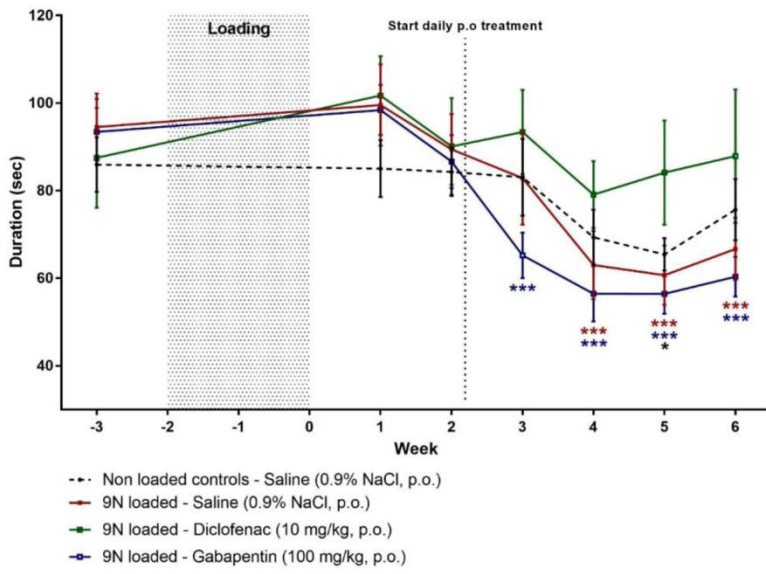## B. Altered weight bearing: diclofenac/gabapentin treatment

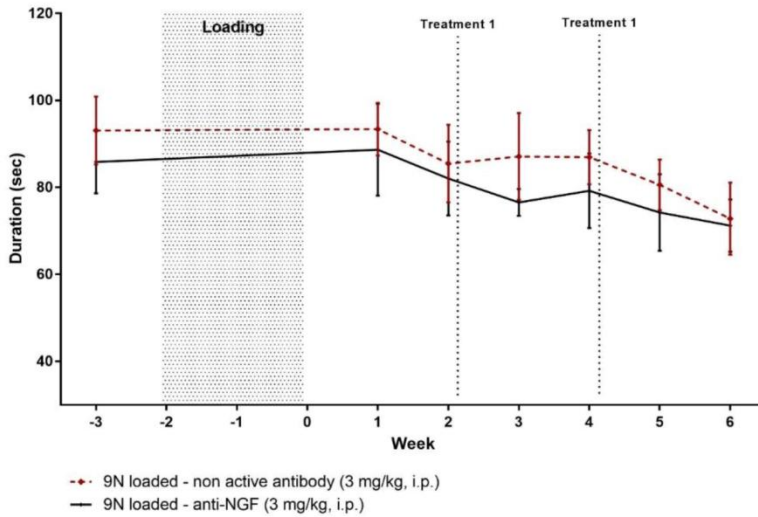## A. Ipsilateral mechanical hypersensitivity: specific anti-NGF mAb treatment

B. Altered weight bearing: specific anti-NGF mAb treatment

A. Motor ability: diclofenac/gabapentin treatment

Legend:
- - - • - - Non loaded controls - Saline (0.9% NaCl, p.o.)
- ——— 9N loaded - Saline (0.9% NaCl, p.o.)
- ——— 9N loaded - Diclofenac (10 mg/kg, p.o.)
- ——— 9N loaded - Gabapentin (100 mg/kg, p.o.)

B. Motor ability: specific anti-NGF mAb treatment

Legend:
- - - • - - 9N loaded - non active antibody (3 mg/kg, i.p.)
- ——— 9N loaded - anti-NGF (3 mg/kg, i.p.)

C. Open field: all treatments