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Central sensitization and neuropathic features of ongoing pain in a rat model of advanced osteoarthritis

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Abstract

Osteoarthritis (OA) pain is most commonly characterized by movement-triggered joint pain. However, in advanced disease, OA pain becomes persistent, ongoing and resistant to treatment with NSAIDs. The mechanisms underlying ongoing pain in advanced OA are poorly understood. We recently showed that intra-articular (i.a.) injection of monosodium iodoacetate (MIA) into the rat knee joint produces concentration-dependent outcomes. Thus, a low dose of i.a. MIA produces NSAID-sensitive weight asymmetry without evidence of ongoing pain while a high i.a. MIA dose produces weight asymmetry and NSAID-resistant ongoing pain. In the present studies, palpation of the ipsilateral hindlimb of rats treated 14 days previously with high, but not low, doses of i.a. MIA produced FOS expression in the spinal dorsal horn. Inactivation of descending pain facilitatory pathways by microinjection of lidocaine within the rostral ventromedial medulla (RVM) induced conditioned place preference (CPP) selectively in rats treated with the high dose of MIA. CPP to intra-articular lidocaine was blocked by pretreatment with duloxetine (30 mg/kg, i.p. at −30 min). These observations are consistent with the likelihood of a neuropathic component of OA that elicits ongoing, NSAID resistant pain and central sensitization that is mediated, in part, by descending modulatory mechanisms. This model provides a basis for exploration of underlying mechanisms promoting neuropathic components of OA pain and for the identification of mechanisms that may guide drug discovery for treatment of advanced OA pain without the need for joint replacement.

Keywords

advanced osteoarthritis; neuropathic pain; duloxetine; central sensitization; descending facilitation

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Introduction

Osteoarthritis (OA) pain is one of the most frequent causes of chronic pain, with symptomatic OA characterized by joint stiffness and pain with movement and joint loading[12, 13, 33]. Some patients develop advanced OA pain characterized as ongoing pain that persists during rest and is resistant to non-steroidal anti-inflammatory drugs (NSAIDs)[12, 13, 33]. In patients with OA pain, joint pathology does not correspond to the degree of pain reported, and the mechanisms driving OA pain are not understood[12, 13, 33]. As chronic OA pain is often inadequately treated, many patients undergo joint replacement therapy in order to alleviate pain[12, 13, 33]. This highlights a need for improved understanding of mechanisms driving NSAID-resistant OA pain to guide development of improved therapies.

Persistent ongoing pain is an important aspect of advanced OA pain that has not been possible to capture in preclinical OA models until recently. Using high dose intra-articular MIA administration to model advanced OA, we demonstrated the presence of both weight asymmetry and ongoing pain[21, 26]. Notably, diclofenac, a NSAID, failed to block the ongoing component of joint pain at a dose that blocked weight asymmetry, consistent with ineffectiveness of NSAIDs on advanced OA pain in patients[26]. We used this preclinical model to explore mechanisms driving NSAID resistant ongoing pain in advanced OA.

Patients reporting moderate to severe pain demonstrate signs of central sensitization such as referred pain and enhanced temporal summation[2, 10, 14, 19, 22, 25, 33, 41]. Expression of the early oncogene, FOS, within the spinal dorsal horn in response to normally non-noxious stimulation such as touch[24, 44] or non-noxious palpation[16, 34] has been used as a marker of the development of spinal sensitization. Another key component of central sensitization is descending pain facilitatory pathways from the rostral ventromedial medulla (RVM)[28]. Transient inactivation of the RVM by administration of lidocaine reverses evoked hypersensitivity and ongoing pain in animals with nerve-injury-induced pain, indicating that both evoked hypersensitivity and persistent ongoing or spontaneous pain are dependent on descending pain facilitatory pathways from the RVM[18, 29, 32, 40]. We therefore examined whether animals in this model of advanced OA pain develop central sensitization.

It has also been proposed that OA patients with moderate to severe ongoing pain may have a neuropathic pain component, leading to suggestions of individualized treatment strategies for these different populations of patients[33, 37, 38]. Recently, duloxetine, a serotonin, norepinephrine reuptake inhibitor (SNRI) was approved by the FDA for treatment of osteoarthritis pain. Antidepressants such as the monoaminergic re-uptake inhibitors duloxetine and milnacipran, are part of the first line of therapies for patients with neuropathic pain[11]. Therefore, we determined whether duloxetine effectively blocks NSAID resistant ongoing pain associated with advanced OA joint pain in this model.

Using a rat model of advanced OA pain, we tested the following hypotheses: 1) central sensitization is observed selectively in the context of ongoing joint pain associated with advanced OA pain and 2) duloxetine, a serotonin-norepinephrine reuptake inhibitor (SNRI) prescribed for neuropathic pain[8, 23, 39], blocks NSAID resistant ongoing joint pain.

Methods

Subjects

Male Sprague Dawley rats weighing 225-300 g at the start of the experiments were housed in an animal care facility at the University of New England, with a 12 hr light/dark cycle. Food and water were available *ad libitum*. All testing was performed in accordance with policies and recommendations of the International Association for the Study of Pain (IASP) and the National Institutes of Health (NIH) guidelines for the handling and use of laboratory animals. All experimental protocols received approval from the Institutional Animal Care and Use Committee (IACUC) of the University of New England.

Bilateral RVM Cannula Implantation

Animals were anesthetized with an injection of ketamine–xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and placed in a stereotaxic apparatus. The skull was exposed and leveled, and bilateral 26-gauge guide cannulas, separated by 1.2 mm, were directed toward the lateral portions of the RVM (anteroposterior, 11.0 mm from bregma; lateral, ±0.6 mm; dorsoventral, 8.5 mm from the skull according to Paxinos and Watson [30]). These coordinates were based on previous studies [18, 32, 40]. The guide cannulas were cemented in place and secured to the skull by small stainless steel machine screws. Animals were allowed to recover 5-7 days post-surgery before intra-articular injections of MIA or saline. Microinjections into the RVM were administered in a volume of 0.5 μl injected through a 33-gauge injector that protruded 1 mm beyond the end of the guide cannula and into fresh tissue to prevent backflow. Injections occurred over a period of 1 min. Cannula placement was verified at the end of the study by microinjection (0.5 μl) of Evans blue dye (50 mg/ml, Sigma Aldrich; Saint Louis, MO, USA). Animals with incorrect cannula placement were used as off-site control animals (Fig 2A).

Intra-articular Injection

Rats were anaesthetized with a 2% isoflurane O_2 mixture and given a single “low” (3 mg) or “high” (4.8 mg) dose of monosodium iodoacetate (MIA, Sigma, USA) through the infra-patella ligament of the left knee in 60 μl saline, corresponding to concentrations of 50 and 80 mg/ml MIA, respectively. Control animals received equivolume sterile saline. Evaluation of pain behaviors occurred 14 days following intra-articular injection of MIA or saline (control).

Weight Asymmetry

Changes in hind paw weight distribution between the left (MIA) and right (contralateral) limbs were utilized as an index of joint discomfort in the MIA-treated knee [21, 26]. An incapacitance tester (Stoelting Co; Wood Dale, IL, USA) was employed for determination of hind paw weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a separate force plate. The force exerted by each hind limb (measured in grams) is determined over a 5-second period. Each data point is the mean of 3 readings. As previously described [21, 26], data are normalized as percent injured/non-injured

weight bearing, such that sensitivity on the injured side is indicated by values <100%; equal weight distribution is indicated by 100%.

Conditioned Place Preference (CPP) Testing

Ongoing pain was assessed using CPP to a chamber paired with RVM or intra-articular lidocaine using a single trial protocol as previously described[26]. Three chamber boxes were used in which there were two pairing chambers with distinctive visual (stripe vs gray), textural (smooth vs textured) and odor (lemonade vs vanilla chapstick applied to the chamber covers farthest from the middle chamber) cues. A brightly lit middle chamber with gray walls and smooth floors divided these chambers. Pre-conditioning (baseline) time spent in each of the boxes was recorded for 15 min on day 13 post MIA injection. Time spent in each chamber was analyzed using ANY-maze (Stoelting Co; Wood Dale, IL, USA), and any rats spending greater than 720 or less than 180 seconds in a chamber were removed from the study (<5% total animals). Chamber assignments were made, with afternoon drug pairings counterbalanced across chambers (half in striped, half in the solid grey chamber).

Conditioning day, day 14 post MIA, RVM lidocaine—Rats received an RVM microinjection (0.5 μl) of saline and were placed immediately (within 2 min) into the appropriate chamber for 30 min. Four (4) hours later, all rats received 0.5 μl RVM microinjection of lidocaine (4% w/v) and were placed immediately into the opposite chamber for 30 min. Testing occurred the following day (D15) wherein the rats were placed drug-free into the CPP boxes with access to all chambers for 15 min. A total of 39 rats were used: 24 treated with 3.0 mg MIA (16 off-site, 8 on-site); 15 treated with 4.8 mg MIA (6 off-site, 9 on-site).

Conditioning day, day 14 post MIA, duloxetine—Rats received systemic (i.p.) administration of saline (vehicle for duloxetine) followed 30 min later by intra-articular administration of saline (200 μl). Four (4) hours later, rats received systemic administration of duloxetine (30 mg/kg, i.p.) or its vehicle (saline, i.p.) 30 min prior to administration of intra-articular lidocaine (200 μl, 4% w/v), a time-point corresponding to effective alleviation of MIA-induced weight asymmetry. Effectiveness of duloxetine was indicated by blockade of lidocaine-induced CPP. This indirect method of examining whether systemic administration of duloxetine was used as the pharmacokinetics of duloxetine's effects are unlikely to support learning through direct pairing. Specifically, the complications of knowing the timing of the onset and peak effect following systemic delivery of these drugs would introduce several potential confounds to interpretation if the drug fails to produce CPP to the chamber (e.g. slow onset of effect is not sufficient to induce pairing, pharmacokinetics associated with systemic drug delivery delays pain relief so that it is not paired with the chamber, etc.). We note that this indirect method of determining whether systemic administration of diclofenac was previously published[26]. A total of 54 rats were used: 18 intra-articular saline, 9 saline and 9 duloxetine; 36 intra-articular MIA (4.8 mg), 17 saline and 19 duloxetine.

Immunohistochemistry analysis of spinal Fos expression

Tissue collection to examine spinal Fos expression occurred 14 days following intra-articular injection of low or high dose MIA or saline (control) into the knee joint. Ipsilateral hindlimbs underwent knee-joint movement in which the thumb and forefinger were moved from the upper leg across the joint down to the foot and then back to the upper leg, creating movement of the knee joint. This occurred at 1 sec intervals across 2 min, 14 days post-treatment, similar to palpation as previously described within a mouse model of cancer-induced bone pain[34]. Animals then underwent intra-cardiac perfusion 2 hrs later. Immediately following perfusion, the spinal cord at L4 was removed and put into 10% formalin overnight. The spinal cord was then moved into a 30% sucrose solution for cryoprotection. Sections were embedded in optimal cutting temperature compound and sliced on a cryostat maintained at −20°C. Spinal cord sections were cut at 30 μm and every 5th section saved for free-floating incubation. Following a wash in 0.1 M phosphate buffered saline (PBS) to clear the optimal cutting temperature compound, sections were incubated in 3% normal goat serum (NGS), (Thermo Scientific, Newington) for 1 hour, followed by 24 hrs in the primary reagent consisting of rabbit anti-c-FOS (Santa Cruz Biotechnology, Santa Cruz), 1% NGS, and 0.1% X-100 triton (Sigma-Aldrich) in 0.1 M PBS, pH = 7.4. After 3 washes in 0.1 M PBS sections are incubated in secondary reagent for 1 hr (Vector, Burlingame) followed by 3 washes in 0.1 M PBS. Sections were incubated in ABC solution for 1 hr (Vector, Burlingame) followed by 3 washes in 0.1 M PB. Sections were then stained using a DAB staining kit (Sigma, St. Louis). Quantification of labeled staining was performed within the ipsilateral dorsal horn, with counts made within the superficial dorsal horn (Laminae I-II) as well as the deep dorsal horn (Laminae III-V) which were added to calculate total FOS within the spinal dorsal horn (Laminae I-V) (see Fig 1A). FOS expression was assessed in 3-4 rats per treatment group, with 6-8 sections counted per spinal cord. All sections were counted by an experimenter blinded to the treatment conditions.

Statistics

Development of weight asymmetry was indicated by a significant shift in weight away from the ipsilateral hindlimb, represented by a decrease in percentage of weight on that hindlimb which was calculated as % shift in weight bearing = (ipsilateral weight/contralateral weight) * 100. These data were analyzed over time by 1-factor repeated analysis of variance (ANOVA) followed by Fisher's least significant difference. Group differences were determined by a 2-factor ANOVA for repeated measures followed by post-hoc analysis between groups using Bonferroni's multiple comparisons test (corrected t-test). For all analyses, $p<0.05$.

For CPP experiments, data were analyzed using 2-factor analysis of variance (ANOVA: pre- vs post-conditioning by treatment group). Post hoc analysis was performed using Bonferroni tests to verify lack of preconditioning differences in time spent in the pairing chambers and comparing post- to preconditioning time spent in the drug-paired chamber. CPP was indicated by an increase in time spent in the drug-paired chamber. If significant CPP was determined, group differences were analyzed using difference scores (post-test – pre-test (BL)) by one-way ANOVA followed by post-hoc comparisons (Dunnett). For all analyses, $p<0.05$.

Results

Knee joint movement-induced FOS expression observed selectively in high dose MIA treated rats

Development of spinal sensitization was determined in saline, low and high dose MIA treated rats by measuring FOS expression within superficial (Lamina I-II) and deep (Lamina III-V) layers of the spinal dorsal horn in rats that underwent knee joint movement of the treated hindlimb (Figure 1A). FOS staining within the spinal dorsal horn verified that knee joint movement produced a robust increase in total FOS expression within the spinal dorsal horn in rats treated with intra-articular injection of high, but not low, dose MIA or saline into the knee joint 14 days earlier (Figure 1B, *$p<0.05$ vs. no movement). Knee joint movement-induced FOS was observed both in superficial lamina (I-II) and deeper lamina (III-V) of high dose MIA treated rats (Figure 1C and D, respectively; *$p<0.05$ vs. no movement).

RVM lidocaine blocks MIA-induced ongoing pain

The role of descending facilitation from the RVM was determined in low and high dose MIA treated rats 14 days following intra-articular injection. Verification of injection site was performed by ink injection (Figure 2A), and data from off-site injections (gray circles) were compared to on-site injections (black circles). Preconditioning time spent in the pairing chambers did not differ in any of the treatment groups ($p>0.05$, Figure 2B). Injection of lidocaine into the RVM produced a significant increase in time spent in the lidocaine-paired chambers compared to pre-conditioning baselines in the high, but not low, dose MIA treated rats (Figure 2B; *$p<0.05$ vs pre-conditioning). Off-site injections failed to increase time spent in the lidocaine paired chamber compared to pre-conditioning baselines (Figure 2B). Comparison of difference scores across groups confirms that RVM lidocaine produced CPP selectively in rats treated with high dose MIA (Figure 2C, *$p<0.05$ vs off-site injection), with no CPP to the RVM lidocaine paired chamber observed in low dose MIA treated rats.

Systemic duloxetine blocks MIA-induced weight asymmetry and ongoing pain

Consistent with previous observations [26], administration of high dose MIA into the intra-articular space of the knee joint produces weight asymmetry within 14 days of administration (Figure 3A, *$p<0.05$ vs BL). Administration of duloxetine (30 mg/kg, i.p.) blocked MIA-induced weight asymmetry within 30 min, and weight asymmetry values returned to pre-duloxetine values between 90 and 120 min (Figure 3A, $^{\#}p<0.05$ vs D14). Equivolume saline (i.p.) failed to block the MIA-induced weight asymmetry.

We determined whether duloxetine (30 mg/kg, i.p.) blocked intra-articular lidocaine-induced CPP during a time period that reversed MIA-induced changes in weight bearing (30 min post-administration) using a previously established protocol [26]. Preconditioning time spent in the pairing chambers did not differ in any of the treatment groups ($p>0.05$, Figure 3B). Rats treated with intra-articular high dose MIA and treated with systemic saline (i.p.) 30 min prior to conditioning to intra-articular lidocaine demonstrated increased post-conditioning time spent in the intra-articular lidocaine paired chamber compared to pre-conditioning times, indicating lidocaine-induced CPP (Figure 3B, *$p<0.05$ vs pre-conditioning). Duloxetine treatment 30 minutes prior to intra-articular lidocaine blocked the post-

conditioning increase in the intra-articular lidocaine-paired chamber in the MIA treated rats (Fig 3B, p>0.05 vs. pre-conditioning). Animals that received intra-articular saline demonstrated equivalent pre- and post-conditioning time spent in the intra-articular lidocaine paired chamber irrespective of systemic saline or duloxetine treatment (Fig 3B, p > 0.05 vs. pre-conditioning). Comparison of difference scores across groups demonstrates that intra-articular lidocaine produced CPP in high dose MIA treated rats that had received systemic saline (i.p.) 30 min prior to intra-articular lidocaine (Figure 3C, *p<0.05 vs saline-saline control group). Duloxetine (30 mg/kg, i.p.) administered 30 min prior to intra-articular lidocaine blocked intra-articular lidocaine induced CPP (Figure 3C, p>0.05 vs vehicle-saline control group).

Discussion

Advanced OA patients suffer from persistent NSAID resistant ongoing pain leading many to undergo joint replacement therapy[12, 13, 33]. It has been proposed that OA patients reporting severe OA pain have neuropathic pain-like symptoms, and develop central sensitization[2, 22, 33]. Previous studies have shown ongoing joint pain in a preclinical model of advanced OA pain that is dependent on afferent input from the knee joint[26]. Using this model, we demonstrate that advanced OA pain is associated with central sensitization. Two aspects of central sensitization, spinal sensitization and descending facilitation, are observed in rats with persistent ongoing pain[29, 42]. These indicators of central sensitization were not observed in rats treated with a “low” dose of MIA, previously demonstrated as failing to induce ongoing pain in spite of development of joint pathology and signs of NSAID sensitive weight asymmetry and tactile hypersensitivity of the hindpaw[26]. In addition, our data demonstrate blockade of MIA induced ongoing pain and weight asymmetry by duloxetine, indicating advanced OA pain may be responsive to treatments that are effective in treating neuropathic pain states[23]. Our results indicate that therapies targeting neuropathic pain may improve the treatment of persistent ongoing pain in patients with advanced osteoarthritis. Further, development of novel compounds targeting molecular pathways implicated in central sensitization may provide improved pain management in advanced OA patients.

Ongoing afferent input has been suggested to result in spinal sensitization[6, 20, 42]. Our data indicate development of central sensitization selectively in the MIA treated rats that demonstrate CPP to pain relief, indicating persistent ongoing joint pain. Previous studies have demonstrated that normally non-noxious stimulation, such as light touch, produces FOS expression within the spinal dorsal horn of rats with CFA induced inflammation[24]. FOS is an early oncogene that is selectively expressed in response to neuronal activation[24], and is not normally observed in the spinal dorsal horn in the absence of noxious stimulation in the absence of injury[24, 34, 44]. Spinal FOS expression in response to normally non-noxious stimulation has been used to measure the development of injury-induced spinal sensitization across models of inflammation-, nerve-injury- and cancer-induced pain states[24, 34, 44]. Our observations demonstrate that movement of the MIA treated knee joint selectively induces FOS expression in the spinal dorsal horn of high dose MIA treated rats. This treatment failed to produce FOS expression in rats treated with intra-articular saline, indicating that this treatment is normally non-noxious, an observation that is consistent with previous reports in

cancer-induced bone pain in mice[34]. The 2 minute knee joint movement did not induce FOS expression in the low dose MIA treated rats, suggesting that this treatment is not sufficiently noxious to induce spinal FOS expression in rats with joint pathology and weight asymmetry but not ongoing pain. Such observations indicate that spinal sensitization develops selectively in rats with persistent ongoing pain. These findings suggest that spinal sensitization develops in the context of persistent afferent input from the MIA treated joint in this model of advanced OA pain.

Another aspect of central sensitization is descending pain facilitation[3, 29]. It is well established that blockade of descending pain facilitatory pathways through inactivation of the RVM is sufficient to block nerve-injury induced thermal and tactile hypersensitivity[28, 29]. In addition, inactivation of the RVM by administration of lidocaine is sufficient to induce CPP in models of nerve injury[18, 32, 40], suggesting that it also effectively blocks nerve-injury induced ongoing pain. Our data demonstrates that blocking descending pain facilitatory pathways from the RVM induces CPP, indicating that blocking descending facilitation produces pain relief in this model of advanced OA pain. Further, RVM lidocaine selectively produced CPP in the high dose MIA treated rats, consistent with our previous observations of development of ongoing pain with this treatment[26]. These data indicate that descending pain facilitatory pathways maintain persistent pain in this model of advanced OA pain.

Several clinical studies have suggested that subpopulations of patients develop central sensitization that is generally associated with increased reported pain severity[22]. Development of central sensitization in this rat model of advanced OA pain is consistent with clinical observations that patients reporting moderate to severe OA pain (≥6/10 on a visual analogue scale) show signs of central sensitization whereas patients with lower pain ratings (<6/10) did not[2]. Patients reporting moderate to severe OA pain demonstrated increased areas of referred hypersensitivity to pressure pain thresholds, enhanced temporal summation and diminished diffuse noxious inhibitory control[2]. Others reported that hip OA patients awaiting hip joint replacement surgery demonstrated bilateral increased sensitivity to innocuous warmth and cold pain, a sign of central sensitization[19]. Another study with hip OA patients awaiting joint replacement therapy demonstrated that cold and punctate stimuli applied to surrounding areas associated with referred pain resulted in enhanced stimulation within the midbrain in areas corresponding to the periaqueductal grey[10]. Whether the development of central sensitization in patients with moderate to severe OA pain is dependent on pain severity regardless of persistent background pain, or whether persistent ongoing pain is a critical component of central sensitization in these patients was unclear. Although pain severity was assessed, whether it was associated solely with movement or was a persistent pain state as described in advanced OA patients[12, 13, 33] was not specifically described within these studies[2, 10, 19]. However, a study in community dwelling older adults with knee OA reported that subjects with symptomatic knee OA experiencing generalized knee pain with radiation had more persistent, severe pain[41]. Together, these studies indicate that a subset of OA patients reporting moderate to severe pain are likely to also report neuropathic pain symptoms and to have more persistent pain.

Our data indicate that duloxetine blocks MIA-induced ongoing pain, suggesting a neuropathic pain component in animals with persistent ongoing pain. These observations are consistent with other studies reporting signs of neuropathic pain during the late phase of MIA-induced osteoarthritis[5, 17, 38]. Several studies demonstrated that MIA-induced late phase hypersensitivity and weight asymmetry are resistant to anti-inflammatory drugs (e.g. naproxen, celecoxib, indomethacin)[17, 31] whereas drugs used to treat neuropathic pain such as anti-epileptics (e.g. gabapentin, pregabalin) and reuptake inhibitors (e.g. amitryptaline, and milnacipran) are effective[5, 17, 38]. Notably, the concentration of MIA used in these studies (2 mg/25 μl corresponding to 80 mg/ml MIA) is the same as that used in our studies (4.8 mg/60 μl corresponding to 80 mg/ml). Further supporting development of nerve injury within this model, ATF-3, a marker of neuronal insult or injury, was increased in the dorsal root ganglion (L4) corresponding to the MIA treated joint 7 and 14 days post-MIA injection[17, 38]. MIA-induced ATF-3 expression was observed in a concentration dependent manner, as 2 μg/25 μl (80 mg/ml) induced more ATF-3 expression compared to rats injected with a lower concentration (1 mg/25 μl, corresponding to 40 mg/ml MIA)[17, 38]. A similar concentration-dependent decrease in intra-dermal nerve fiber density was observed in the ipsilateral hindpaw of MIA treated rats, further supporting the hypothesis of neuronal damage at the 80 mg/ml concentration[38]. Collectively, these data indicate that MIA administration at a concentration of 80 mg/ml produces signs of neuropathic pain. Moreover, these studies indicate that such pain states are more responsive to pharmacological treatments effective in neuropathic pain patients such as pregabalin or SNRIs such as duloxetine than treatments effective in inflammatory pain states, such as NSAIDs (e.g. diclofenac). Such findings are consistent with observations of neuropathic pain like characteristics in some patients with OA[33, 37]. Further, our observations that rats with ongoing pain that is responsive to duloxetine develop central sensitization is consistent with several studies indicating that signs of central sensitization correlate with symptoms associated with neuropathic pain[14, 15, 25].

Antidepressants such as the monoaminergic re-uptake inhibitors duloxetine and milnacipran have been demonstrated to block pain independent of their effects on mood[1, 11]. The pain alleviating effects of these antidepressants are thought to be due to increased synaptic availability of both serotonin and norepinephrine. Supporting this, selective serotonin reuptake inhibitors (SSRIs) have been found to be less effective in ameliorating pain compared to serotonin/norepinephrine reuptake inhibitors[9]. Both norepinephrine and serotonin have been implicated in descending pain modulation[27]. Norepinephrine has been indicated in descending pain inhibition through activity at spinal noradrenergic α2 receptors[43]. Serotonin has been demonstrated to have both pain facilitatory activity at spinal 5-HT3 receptors[35, 36] and pain inhibitory activity at spinal 5-HT7 receptors[4, 7]. Consistent with observations that SNRIs show some efficacy in chronic neuropathic pain states, our data demonstrate that duloxetine blocked spontaneous pain at a dose and time-point demonstrating peak effectiveness against weight asymmetry.

In summary, we demonstrate that development of persistent ongoing joint pain in this model of advanced osteoarthritis pain is associated with development of central sensitization and responsive to therapies used to treat neuropathic pain in patients. Notably, duloxetine has recently been approved for treatment of osteoarthritis pain. Our studies indicate that

duloxetine may be effective in patients with persistent, NSAID resistant osteoarthritis pain, and may diminish the number of patients requiring joint replacement surgery. Further, our studies indicate that development of drugs that target molecular mechanisms associated with development of central sensitization may provide alternative treatment options for patients resistant to current therapeutic options.

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Perspective

Difficulty in managing advanced osteoarthritis pain often results in joint replacement therapy in these patients. Improved understanding of mechanisms driving NSAID resistant ongoing OA pain may facilitate development of alternatives to joint replacement therapy. Our findings suggest central sensitization and neuropathic features contribute to NSAID resistant ongoing OA joint pain.

Highlights

- Central sensitization is selectively observed in rats with ongoing knee joint pain
- Duloxetine, a therapy used for neuropathic pain, blocks ongoing knee joint pain
- Results demonstrate reverse translation from clinic to preclinical model of OA
- Suggest use of compounds targeting neuropathic pain and central sensitization

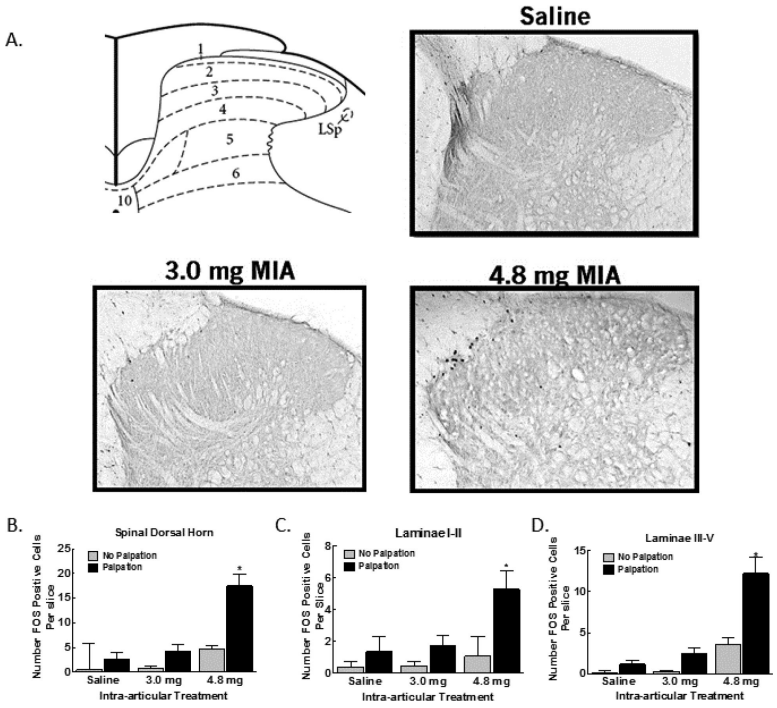

Figure 1. Knee joint movement-induced FOS expression observed selectively in 4.8 mg MIA treated rats

A) Representative images depicting FOS expression in each of the treatment groups. Spinal map from The Rat Brain in Stereotaxic Coordinates, Fourth Edition, Paxinos and Watson[30]. **B)** Movement of the knee joint (2 min, 1 movement/sec) produced a robust increase in FOS expressing cells within the spinal cord dorsal horn in rats treated with intra-articular injection of 4.8 mg MIA, but not 3.0 mg MIA or saline into the knee joint 14 days earlier. **C)** Movement of the knee joint increased FOS expression within the superficial dorsal horn (Laminae I&II). **D)** Knee joint movement increased FOS expression within the deep dorsal horn (Laminae III-V). Graphs represent Mean ± SEM, counts represent 6-8 sections across 3-4 rats per group.

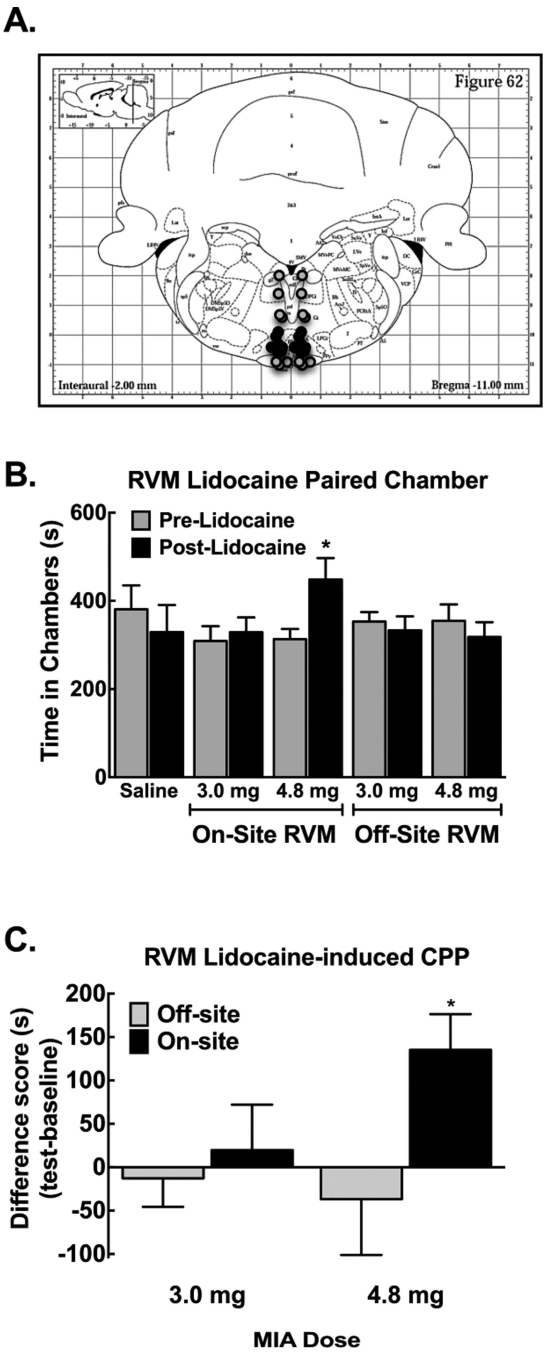

Figure 2. RVM Lidocaine blocks MIA-induced ongoing pain

A) Diagram illustrating verification of bilateral cannulation of the RVM. Map from The Rat Brain in Stereotaxic Coordinates, Fourth Edition, Paxinos and Watson, 1998. Hits (dark colored circles) and misses (light colored circles) are illustrated. All misses were added to the off-site groups. **B)** Pre- and post- conditioning time spent in the lidocaine paired chamber demonstrates that only 4.8 mg MIA treated rats with on-site RVM lidocaine injections demonstrated increased time spent in the lidocaine paired chamber, *$p<0.05$ vs pre-lidocaine. **C)** Difference scores verify that RVM lidocaine produced preference

selectively in rats treated with 4.8 mg MIA. No preference was observed in saline or 3.0 mg MIA treated rats, *p<0.05 vs off-site controls. Off-site injections failed to produce CPP. Graphs represent Mean ± SEM, n=6-16.

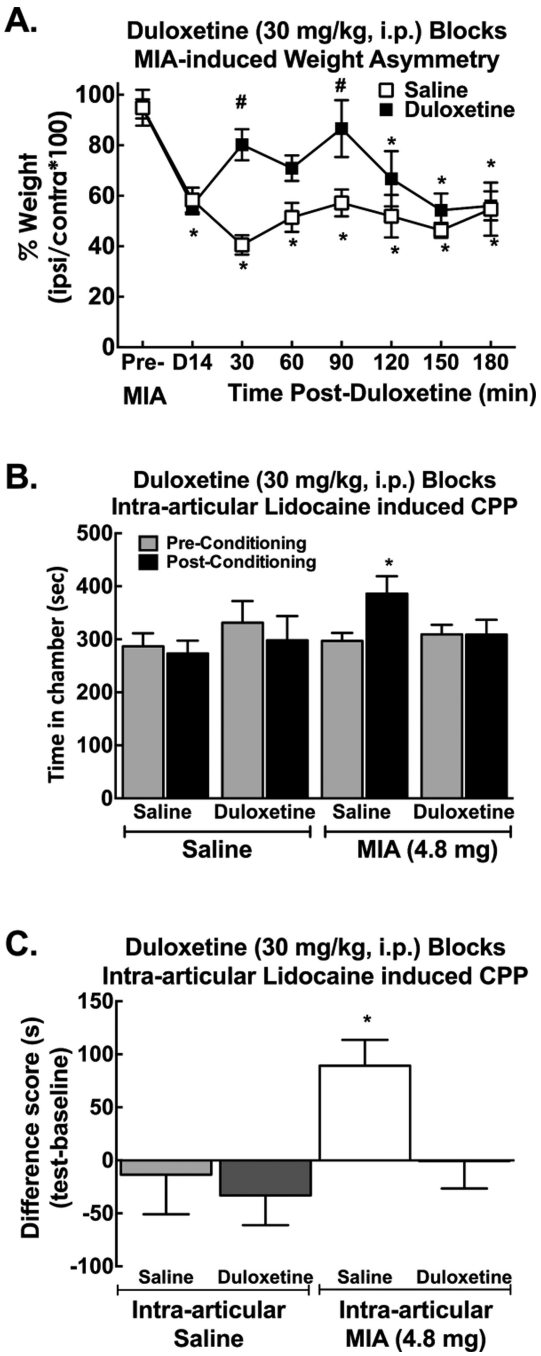

Figure 3. Systemic duloxetine blocks MIA-induced weight asymmetry and ongoing pain

A) Intra-articular MIA (4.8 mg/60 μl) produced weight asymmetry within 14 days of administration. Systemic duloxetine (30 mg/kg, i.p.) blocked MIA-induced weight asymmetry. Systemic saline failed to block the MIA-induced weight asymmetry. *p<0.05 vs Pre-MIA, #p<0.05 vs D14 (pre-duloxetine), n=9-19. **B)** Pre- and post-conditioning time spent in the intra-articular lidocaine paired chamber demonstrates that 4.8 mg MIA treated rats that received systemic saline 30 min prior to intra-articular lidocaine, increased time spent in the lidocaine paired chamber, *p<0.05 vs pre-conditioning. Systemic duloxetine (30

mg/kg, i.p.) blocked post-conditioning increase in the intra-articular lidocaine paired chamber. Rats treated with intra-articular saline failed to demonstrate increased time spent in the lidocaine-paired chamber irrespective of systemic (i.p.) saline or duloxetine treatment. n= 9 (intra-articular saline) and 17-19 (intra-articular MIA). **C)** Difference scores demonstrate that intra-articular lidocaine produced CPP in MIA (4.8 mg) treated rats that had been treated with saline (i.p.) 30 min prior to intra-articular lidocaine. Duloxetine (30 mg/kg, i.p.) blocked intra-articular lidocaine-induced CPP. *$p<0.05$ vs saline/saline controls. All graphs are mean ± SEM.