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Monosodium iodoacetate-induced osteoarthritis produces pain-depressed wheel running in rats: Implications for preclinical behavioral assessment of chronic pain

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ABSTRACT

Pain stimulates some behaviors (e.g., withdrawal responses) and depresses other behaviors (e.g., feeding and locomotion). We are developing methods for testing candidate analgesics using measurements of pain-depressed behaviors. Such assays may model important aspects of clinical pain and complement traditional procedures that measure pain-stimulated behaviors. The present study characterized the effects of a chronic pain manipulation (monosodium iodoacetate (MIA)-induced osteoarthritis) on wheel running in rats. Rats had 24 h voluntary access to running wheels. Duration of running wheel acquisition was manipulated such that rats had either 21 or 7 days of running wheel access prior to MIA administration. Wheel running was monitored for an additional 21 days following MIA administration. MIA produced concentration- and acquisition length-dependent decreases in wheel running. Parallel experiments demonstrated that MIA produced concentration-dependent tactile allodynia and shifts in hind limb weight bearing. MIA was differentially potent across assays with a potency rank: weight-bearing ≥ von Frey > running wheel. MIA produced greater depression of wheel running in rats with relatively high baseline running rates compared to rats with relatively low baseline running rates. The differential potency of MIA across assays and apparent rate-dependent effects in running wheels may impact our traditional interpretations of preclinical nociceptive and antinociceptive testing.

1. Introduction

Chronic pain continues to be a major public health problem, in part due to incomplete efficacy and intolerable side effects associated with currently available analgesic drugs. Reliable and predictive preclinical measures of pain and antinociception are an important factor in the development of better analgesic drugs. Although clinical pain is typically associated with pain-stimulated behavior (e.g., behavior that increases in frequency or intensity following a noxious stimulus), spontaneous pain behavior (e.g., behavior that emerges in the absence of any clear noxious stimulus), and pain-depressed behavior (e.g., normally adaptive behavior that decreases in frequency or intensity following a noxious stimulus), preclinical assays of pain and analgesia rely almost exclusively on measures of pain-stimulated behavior (Le Bars et al, 2001; Mogil, 2009). There are at least two limitations to this approach. First, although assays of pain-stimulated behavior are thought to be predictive of many acute pain states, they may not be adequate models of clinical chronic pain (e.g., sustained inflammatory and/or neuropathic pain states). In support of this, assessment of chronic pain in clinical medicine (both human and veterinary) relies heavily on measurement of pain-*depressed* behavior (Flecknell et al., 1999; Goodwin and Bajwa, 2004; Martin et al., 2004; Wittink et al, 2004). Consistent with this approach, chronic pain patients report that pain interferes with their ability to perform daily tasks, ability to walk, and ability to exercise (Lazarus and Newmann, 2001). A second limitation of using pain-stimulated behavior is that candidate analgesic drugs may decrease pain-stimulated behavior by producing motor effects that impair the subject's ability to respond, thus producing a false positive analgesic result (Stevenson et al., 2006).

One approach to addressing each of these limitations is to incorporate measures of pain-depressed behaviors into preclinical testing batteries (Negus et al, 2006). The inclusion of measurements of pain-depressed behaviors would permit a more comprehensive assessment of the impact of pain on the organism, and provide a complementary approach for assessing antinociceptive efficacy. Certainly, assays of pain-depressed behavior are not without their own limitations. For example, drugs may increase pain-depressed behaviors by non-selective stimulation, producing a false positive analgesic effect (Negus et al, 2006). However, it is unlikely that a candidate analgesic would produce a false positive effect in an assay of pain-stimulated behavior (via non-selective motor suppression) and a false positive in an assay of pain-depressed behavior (via non-

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selective motor stimulation). Thus, combined use of pain-stimulated and pain-depressed measures may yield a preclinical strategy with enhanced predictive validity.

Our laboratories recently conducted studies using acute pain manipulations to develop an approach to the study of pain-depressed behaviors in rodents (Pereira Do Carmo et al., 2009; Stevenson et al, 2006, 2009). Assays of pain-depressed behavior may have more general applicability if they were also sensitive to manipulations thought to model longer lasting (i.e., chronic) pain states (pain lasting months to years in humans and weeks to months in laboratory animals). Toward that end, the present study represents an assessment of the ability of a chronic pain manipulation (monosodium iodoacetate-induced osteoarthritis) to depress wheel running. Intra-articular injection of monosodium iodoacetate (MIA) was chosen because it has been shown to produce long-lasting osteoarthritis (OA) of the knee joint in rodents (Bove et al, 2003; Guingamp et al., 1997). This model of experimentally induced OA has good face validity because OA of the knee is the most common form of OA in humans, and the associated pain, inflammation and degeneration of joint cartilage closely parallel symptoms in the human population (Clarke et al., 1997). Wheel running was chosen as the behavioral endpoint due to (a) the ability to automate data collection, and (b) the clinical relevance of the measured behaviors to chronic pain states and activity in humans.

Assay development proceeded as follows: 1) identification of conditions under which wheel running occurred at reliable and high rates, 2) characterization of a chronic osteoarthritis pain manipulation (intra-articular monosodium iodoacetate; MIA) that reliably depressed wheel running and 3) assessment of the effects of varying the duration of running wheel acquisition on subsequent MIA-depressed wheel running. Effects of MIA on wheel running were compared to the effects of MIA on tactile sensitivity using von Frey monofilaments (a traditional pain-stimulated measure) and hind limb weight bearing (often interpreted as a measure of spontaneous pain).

2. Materials and methods

2.1. Subjects

Adult male Sprague Dawley rats (200–300 g at the start of the experiment; Harlan, Indianapolis, IN) were used for all studies. Rats were housed in groups of two to three in standard Plexiglas containers with food and water available ad libitum. (Note: running wheel rats were group housed upon arrival and then moved to individual cages with running wheels attached.) Animals were maintained in a temperature and humidity controlled colony on a 12-h light/dark cycle (lights on at 8:00). All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. The University of New England Institutional Animal Care and Use Committee (IACUC) approved all protocols involving animals.

2.2. Assay of MIA-stimulated mechanical allodynia

Tactile allodynia was assessed using von Frey monofilaments. All von Frey experiments were conducted with separate groups of rats from running wheel experiments. Rats were removed from their home cages and placed in a Plexiglas cage with a wire mesh bottom. The rats were allowed to acclimate for 15 min (or until exploratory and grooming behavior declined to a level compatible with behavioral testing). Von Frey monofilaments were applied to the mid-plantar left hind paw (ipsilateral side of MIA injection) with a series of monofilaments that ranged in stiffness from 0.4 to 15 g (0.4, 0.6, 1, 2, 4, 6, 8, 15 g). Filaments were applied once for 5 s with inter-stimulus intervals of 1 min. Rats were tested using the up–down method (Chaplan et al., 1994). Briefly, rats were tested beginning with the 2 g monofilament. If the rat did not emit a positive response, the next highest filament in the sequence was tested until the rat showed a positive response. If the rat did respond to the 2 g monofilament, the next lowest filament was used until the rat stopped emitting a positive response. A positive response was defined as a rapid withdrawal of the left hind paw or licking of the paw. The first day of testing provided a baseline measure of tactile sensitivity. The next day, rats were removed from their home cage and injected with vehicle or a range of concentrations (0.32–3.2 mg) of intra-articular monosodium iodoacetate (MIA) into the left hind knee and returned to their home cages and allowed to recover. Rats were then tested with von Frey monofilaments on post-injection days 3, 7, 14 and 21. Testing was conducted between 12 pm and 5 pm.

2.3. Assay of MIA-induced shifts in weight bearing

An Incapacitance tester (Columbus Instruments, Columbus, OH) was used to determine hind paw weight distribution. Rats were placed in a custom-made, angled Plexiglas chamber so that each hind paw rested on a separate force plate. The change in hind paw weight distribution was automatically calculated by the Incapacitance tester (the difference in the amount of weight (g) between the left and right limbs). Essentially, the apparatus calculates an average weight distribution over the span of 5 s, and three recordings are taken for each rat. All three recordings are then automatically averaged and a mean score is displayed. The primary dependent measure was % weight on ipsilateral hind paw and was determined by the following formula:

force (g) of left hind paw ÷ (force (g) of left hind paw
+ force (g) of right hind paw)*100.

After habituation to the Plexiglas chamber, baseline recordings were determined. The next day, rats were removed from their home cage and injected with a range of concentrations (0.32–3.2 mg) of intra-articular monosodium iodoacetate (MIA) or saline into the left hind knee, returned to their home cages, and allowed to recover. Rats were then tested in the Incapacitance tester on post-injection days 3, 7, 14 and 21. Testing was conducted between 12 pm and 5 pm. Test trials for each rat were 30–60 s with an average session length of approx. 45 min for n = 16 rats.

2.4. Assay of MIA-depressed wheel running

Wheel running was measured using an activity wheel monitoring system (Lafayette Instruments, Lafayette, IN). In running wheel experiments, rats were singly housed in a chamber that contained an activity wheel. Each rat had 24 h voluntary access to its own running wheel for the duration of the experiment. The total distance traveled (meters) in the wheels by each rat was used as the primary dependent measure. Each running wheel experiment consisted of three phases: a) an acquisition phase of 21 or 7 days, b) an osteoarthritis induction phase consisting of a single intra-articular injection of either saline, 0.32, 1, or 3.2 mg monosodium iodoacetate into the left hind knee, and c) a post-injection observation phase which lasted 21 days.

2.4.1. Acquisition phase

The length of the Acquisition phase (number of days access to wheels before saline or MIA injection) was manipulated in order to generate an efficient protocol in which high and stable levels of wheel running were established and subsequently depressed by MIA. Two different experiments with distinct acquisition durations were completed: Experiment I consisted of a 21-day acquisition period and Experiment II consisted of a 7-day acquisition period. The 21-day acquisition was initially chosen because it yielded high rates of

running, with running stabilizing between days 17–21. The 7-day acquisition was chosen in an effort to stream-line the experimental protocol and increase throughput.

2.4.2. Osteoarthritis induction phase

A single intra-articular injection of MIA (0.32–3.2 mg) into the left hind knee was administered to induce a localized arthritis of the knee joint.

2.4.3. Post-MIA phase

The post-MIA phase consisted of a 21-day observation phase in which the effects of MIA-induced osteoarthritis or saline on wheel running were recorded daily.

2.5. Histology

On MIA-post day 22, rats were sacrificed and MIA- or saline-injected knee joints (including distal femur and proximal tibia) were stored in 10% neutral buffered formalin (Sigma-Aldrich, product # HT5011) for a minimum of 48 h. All bones were stored in separate vials. Bones were trimmed to a maximum length of 22.0 mm and any remaining muscular tissue was removed. Bones were rinsed with phosphate-buffered saline, PBS for 20 min, in triplicate, and then rinsed with ddH2O for 20 min, in triplicate. After the final rinsing with ddH2O, bones were decalcified in a 10% EDTA solution with pH buffered between 7.2 and 7.4 for 15 days (solutions changed daily), and subsequently embedded in paraffin. Sagittal 4 μm sections, starting at 1500 μm with 750 μm gaps, were prepared and stained with Hematoxylin and Eosin (H&E).

2.6. Data analysis

The primary dependent variable of the von Frey test was withdrawal threshold (in grams). The primary dependent variable of the weight-bearing test was % weight on ipsilateral (injured) hind paw. The two dependent variables for the running wheel experiments were total distance traveled in the running wheels (meters) during the dark cycle, and % control of mean baseline wheel running during dark cycle. Baseline for running wheel Experiment I was defined as the mean of acquisition days 17–21, and baseline for running wheel Experiment II was defined as the mean of acquisition day 7. Statistical analysis was accomplished with one- or two-factor ANOVA as appropriate. A significant one-way or two-way ANOVA was followed by the Duncan post hoc test. Significance was set *a priori* at $p \leq 0.05$.

3. Results

3.1. MIA-stimulated mechanical allodynia

Fig. 1A shows the effects of MIA (0.32–3.2 mg) on withdrawal thresholds from von Frey monofilaments. Baseline levels of withdrawal threshold averaged approximately 13.8 g. Relative to controls, injection of saline did not produce any change in withdrawal threshold across the 21-day post-injection phase. MIA, in contrast, produced a concentration-dependent decrease in withdrawal threshold (e.g., increase in mechanical sensitivity or allodynia) with 1 mg and 3.2 mg MIA producing similarly robust and sustained effects. For example, the peak effect of 1 mg MIA was 1.7 g, and occurred on post-injection day 14, whereas the peak effect of 3.2 mg MIA was 1.8 g, and occurred on post-injection day 21.

3.2. MIA-induced shifts in weight-bearing

Fig. 1B shows the effects of MIA (0.32–3.2 mg) on weight bearing. During baseline recordings of weight bearing, rats distributed approx. 50% of their weight on both ipsilateral and contralateral sides, yielding a balanced, stable posture. Relative to baseline, injection of saline did not alter posture. MIA, however, produced a concentration-dependent decrease in weight on the ipsilateral (injured) hind paw with 3.2 mg MIA producing the most robust and sustained shifts in posture (peak effect being 22% of weight on injured hind limb on post-injection day 3).

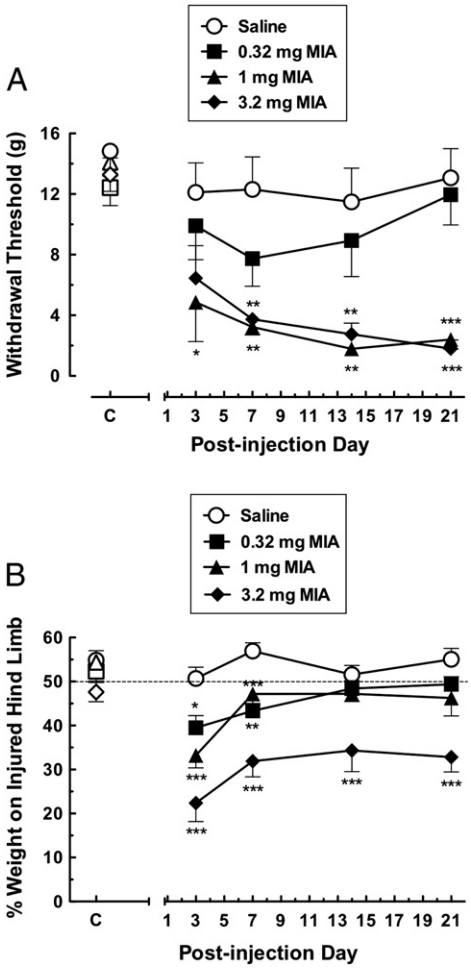

Fig. 1. Effects of concentration and post-injection day on monosodium iodoacetate (MIA)-stimulated mechanical allodynia and MIA-induced shifts in hind limb weight bearing. All points show mean (±SEM) data, with n = 8 per group. Fig. 1A Abscissa: Post-injection day. Data above point "C" represents withdrawal thresholds under control conditions (prior to MIA administration). Ordinate: Withdrawal threshold from von Frey monofilaments (in grams). Fig. 1B Abscissa: Post-injection day. Data above point "C" represents distribution of hind limb weight under control conditions (prior to MIA administration). Ordinate: % weight on ipsilateral (injured) hind paw. For Fig. 1A, separate ANOVAs for post-injection days 3, 7, 14 and 21 revealed significant differences (F values of 2.79, 7.77, 7.14, and 17.02, respectively). For Fig. 1B, separate ANOVAs for post-injection days 3, 7, 14 and 21 revealed significant differences (F values of 14.16, 17.74, 7.69, and 8.18, respectively). Post-hoc analyses revealed significant differences from saline control levels ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

3.3. MIA-depressed wheel running [Experiment I]

Fig. 2 shows the effects of MIA on wheel running during Experiment I. Fig. 2A shows a line graph depicting the protocol for Experiment I. The duration of acquisition was 21 days. OA induction involved intra-articular administration of 3.2 mg monosodium iodoacetate (MIA) into the left hind knee. The post-MIA injection phase lasted 21 days. Fig. 2B shows mean total distance traveled in the running wheels (m = meters) during the 21-day acquisition phase, before rats were separated into saline or MIA runners. Wheel running

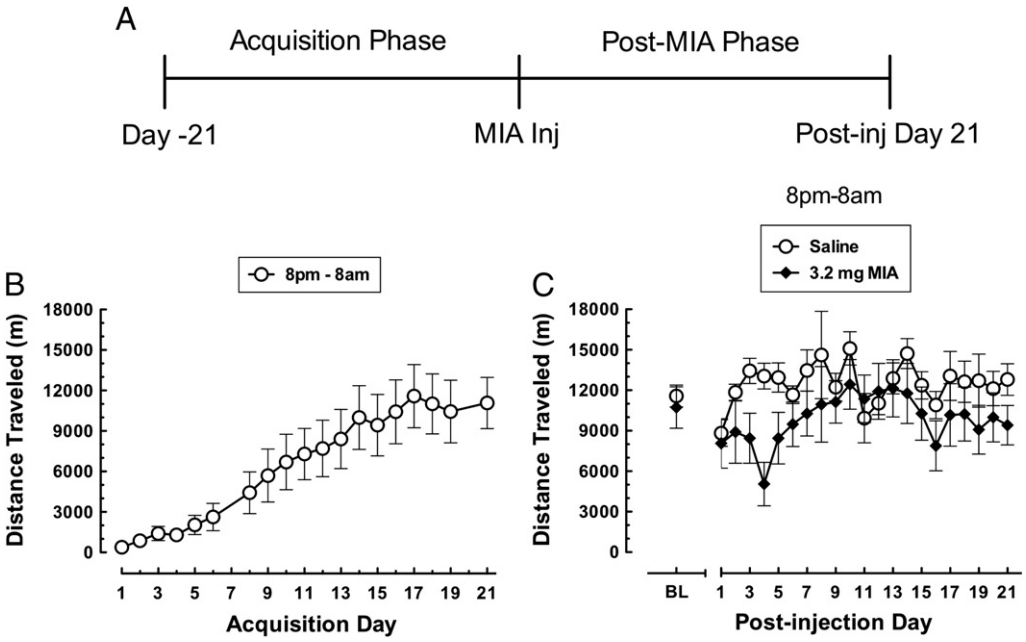

Fig. 2. Running wheel Experiment I. Panel A shows the three components of Experiment I are a 21 day acquisition phase, MIA administration, and a 21 day post-MIA phase. Panel B shows mean (±SEM) distance traveled (m) in the running wheels during the 21-day acquisition phase, during the dark cycle (8 pm–8 am). Abscissa: Acquisition day. Ordinate: Total distance traveled (m). Panel C shows the effects of 3.2 mg MIA on mean (±SEM) total distance traveled in the running wheels during the 21-day post-MIA phase, with n = 5 for Saline group and n = 8 for MIA group. Abscissa: Post-injection day. Data above "BL" represents the mean distance traveled during acquisition days 17–21. Ordinate: Total distance traveled (m). Two-factor ANOVA showed a significant main effect for MIA concentration (saline vs. 3.2 mg), $F(1,20) = 18.59$, $p < 0.001$; but not for Time (Day), $F(1,20) = 0.83$, $p > 0.05$, or Interaction, $F(2,20) = 0.45$, $p > 0.05$. Post-hoc analyses revealed no significant differences between saline and MIA runners at any time point.

increased from acquisition days 1–17 and then remained stable thereafter (Note that a power outage occurred on acquisition days 7 and 20 and no data were recorded on those days). The mean total distance traveled at acquisition day 21 was 11,000 m. Fig. 2C shows the effects of MIA during the post-MIA injection phase. Mean baseline wheel running for saline rats was 11,500 m and baseline wheel running for MIA rats was 10,900 m. Relative to saline controls, MIA (3.2 mg) produced a time-dependent depression of running during post-injection days 3–5. There were no significant differences between saline and MIA runners from post-injection days 1–21. In an effort to produce a more robust and sustained depression of running, the acquisition phase was shortened (see Experiment II, below).

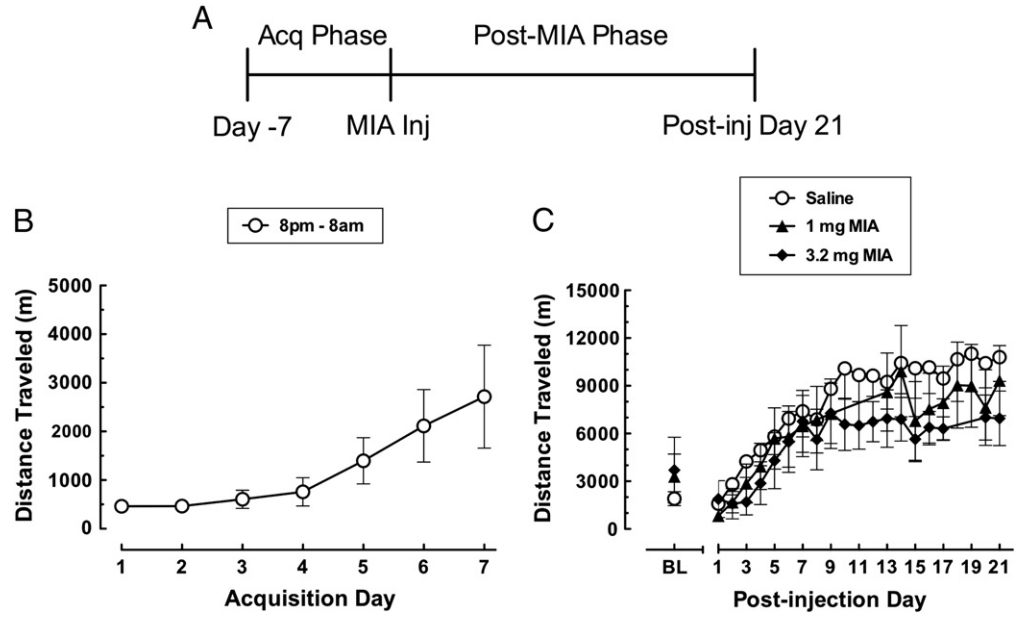

Fig. 3. Running wheel Experiment II. Panel A shows the three components of Experiment II are a 7 day acquisition phase, MIA administration, and a 21 day post-MIA phase. Panel B shows mean (±SEM) distance traveled (m) in the running wheels during the 7-day acquisition phase, during the dark cycle (8 pm–8 am). Abscissa: Post-injection day. Ordinate: Total distance traveled (m). Panel C shows the effects of 1 mg and 3.2 mg MIA on mean (±SEM) total distance traveled in the running wheels during the 21-day post-MIA phase, with n = 8 for Saline group, n = 6 for 1 mg MIA group, and n = 5 for 3.2 MIA group. Abscissa: Post-injection day. Data above "BL" represents the mean distance traveled during acquisition day 7. Ordinate: Total distance traveled (m).

3.4. MIA-depressed wheel running [Experiment II]

Fig. 3 shows the effects of MIA on wheel running during Experiment II. Fig. 3A shows a line graph depicting the protocol for Experiment II. The duration of acquisition was 7 days. OA induction involved intra-articular administration of either 1 mg or 3.2 mg monosodium iodoacetate (MIA) into the left hind knee, and the post-MIA injection phase lasted 21 days. Fig. 3B shows mean total distance traveled in the running wheels during the 7-day acquisition phase, before rats were separated into saline or MIA runners. Wheel running increased steadily from days 2 to 7. The mean total distance traveled at acquisition day 7 was 2500 m. Fig. 3C shows the effects of MIA during the post-MIA phase. Mean baseline wheel running for saline rats was 2000 m and baseline wheel running for MIA rats was 4000 m. Relative to baseline, saline rats demonstrated a steady increase in total distance traveled from post-injection days 1–9, with running leveling off for the remaining post-injections days 10–21. Relative to baseline, MIA (1 and 3.2 mg) rats showed a decrease in total distance traveled from post-injection days 1–3, and levels of wheel running recovered to near-baseline levels by post-injection day 4. For the 1 mg MIA group, wheel running steadily increased through post-injection day 14, with running leveling off for the remaining post-injection days 15–21. For the 3.2 mg MIA group, wheel running steadily increased through post-injection day 9, with running leveling off for the remaining post-injection days 10–21. (Note that power outages occurred on days 10–12 for the 1 mg group and 18–19 for the 3.2 mg group; no data were recorded on those days) To account for the different baseline levels of running between groups, the y-axis of Fig. 3C was transformed to %mean baseline for both saline and MIA runners (see Fig. 4). Fig. 4 shows the time course of effects produced by saline and the two doses of MIA (1 and 3.2 mg) with each y-axis transformed to %mean baseline (of total distance traveled), in order to normalize both saline and MIA baseline wheel running. Fig. 4A shows that a dose of 1 mg MIA did not produce any significant decreases in wheel running relative to saline controls. In contrast, Fig. 4B shows that 3.2 mg MIA produced a robust and sustained decrease in wheel running, relative to saline controls. A sustained separation between saline and MIA-treated rats was evident from post-injection days 10–21.

3.5. MIA-induced histopathological changes to bone and cartilage

Tests of mechanical allodynia and weight bearing are two established preclinical measures of nociception (Chaplan et al., 1994; Bove et al., 2003). However, the effects of nociceptive manipulations on locomotor endpoints are less well established as measures of preclinical pain (Stevenson et al., 2009). In an effort to demonstrate that MIA-induced depression of wheel running was concomitant with changes to joint morphology typified by OA pain, Fig. 5 shows the effects of saline and MIA on the articular cartilage as well as underlying subchondral bone for rats with 24 h access to running wheels in Experiment II. Saline-injected runners showed healthy femur (a) and tibia (d) sections. In contrast, 1 mg MIA-injected runners showed degradation of articular cartilage and hypertrophy in the subchondral regions for femur (b) and tibia (e). The highest concentration of 3.2 mg MIA produced the most robust hypertrophy and remodeling of femur (c) and tibia (f). Numbers correspond to approx. thickness in μm. Femur panels a–c are at 4× magnification. To permit close-up images of tibia articular cartilage, panels d–f are at 10× magnification.

3.6. MIA-depressed wheel running as a function of baseline running rates

Fig. 6 shows the efficacy of MIA to depress wheel running in rats with relatively low baseline rates (panel A) vs. rats with relatively high baseline rates (panel B). To determine if levels of running at baseline affected the ability of MIA to depress running, rats were divided into two groups, namely, low runners and high runners. Low runners were operationally defined as rats that ran an average of 1000 m/day at baseline (range 415–1500 m), whereas high runners were operationally defined as rats that ran an average of 6000 m/day at baseline (range 3500–9600 m). Fig. 6A shows the effects of saline and 3.2 mg MIA in low runners. For low runners, there were no significant differences between MIA and saline runners on any of the post-injection days. Fig. 6B shows the effects of saline and 3.2 mg MIA in high runners. For high runners, MIA produced significant depression of running at all post-injection days assessed (PD 3, 7, 14, and 21). Also notable is the finding that saline low runners showed a steady increase in running across post-injection days, whereas saline high runners showed an initial increase through PD 7 and then stabilized running across PD 7, 14, 21.

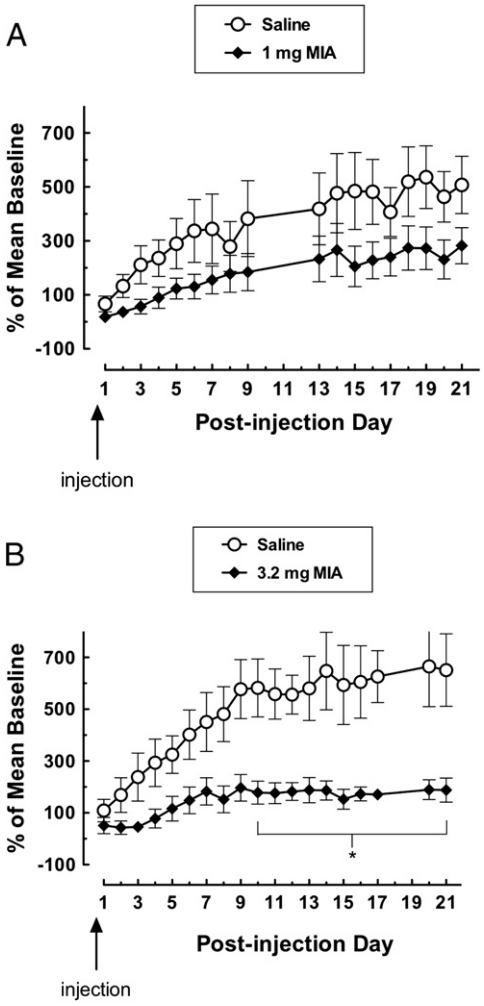

Fig. 4. % of mean (±SEM) baseline for running wheel Experiment II with n = 8 for Saline group, n = 6 for 1 mg MIA group, and n = 5 for 3.2 mg MIA group. Panel A shows y-axis transformation of Fig. 3C to %mean baseline for 1 mg MIA. Panel B shows y-axis transformation of Fig. 3C to %mean baseline for 3.2 mg MIA. Abscissa: Post-injection day. Ordinates: % of mean baseline wheel running. *Significantly different from control levels ($p<0.05$). Two-factor ANOVA showed a significant main effect for MIA concentration (saline vs. 3.2 mg), $F(1,20) = 10.86$, $p<0.05$, Time (Day), $F(1,20) = 13.06$, $p>0.0001$, and Interaction, $F(2,20) = 4.63$, $p<0.001$. Post-hoc analyses revealed significant differences from saline control levels from post-injection days 10–21 (*$p<0.05$).

4. Discussion

The present paper represents our continued interest in and development of measures of pain-depressed behavior. Previous

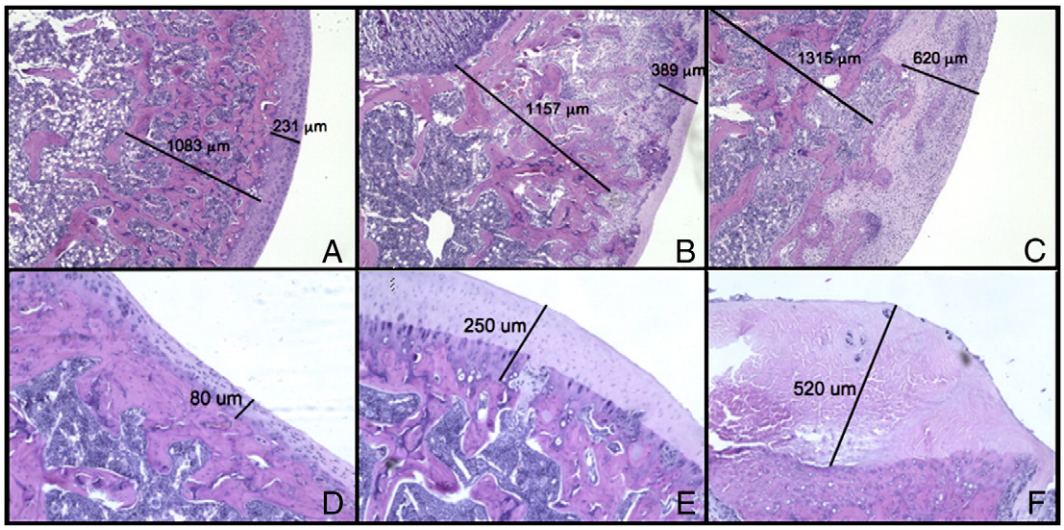

Fig. 5. Hematoxylin and Eosin (H&E) staining of femur and tibia bones. Panels A–C represent H&E staining of the distal end of the femur for saline, 1 mg MIA and 3.2 mg MIA runners, respectively. Panels D–F represent H&E staining of the tibial plateau for saline, 1 mg MIA and 3.2 mg MIA runners, respectively. Numbers represent approx. thickness in µm.

studies from our laboratories have established that an acute pain state (i.p. acetic acid) can depress both feeding and locomotor activity, and that opioid analgesics can restore both pain-depressed behaviors (Pereira Do Carmo et al., 2009; Stevenson et al, 2006, 2009). To facilitate translation of these preclinical measures to clinical chronic pain conditions, the present study assessed the ability of a well-characterized chronic pain manipulation (intra-articular monosodium iodoacetate-induced osteoarthritis) to depress wheel running in rats. As a comparison, the effects of monosodium iodoacetate (MIA) were also evaluated in a standard test of tactile sensitivity (a traditional pain-stimulated behavior) as well as an assay of hind limb weight bearing (a putative measure of spontaneous pain behavior). The main findings were that MIA demonstrated differential potency and efficacy in each of the three assays, namely, wheel running, von Frey, and weight bearing. Specifically, MIA (0.32–3.2 mg) produced a time- and concentration-dependent decrease in wheel running. Relative to wheel running, MIA was more potent in producing tactile hypersensitivity and shifts in hind limb weight bearing.

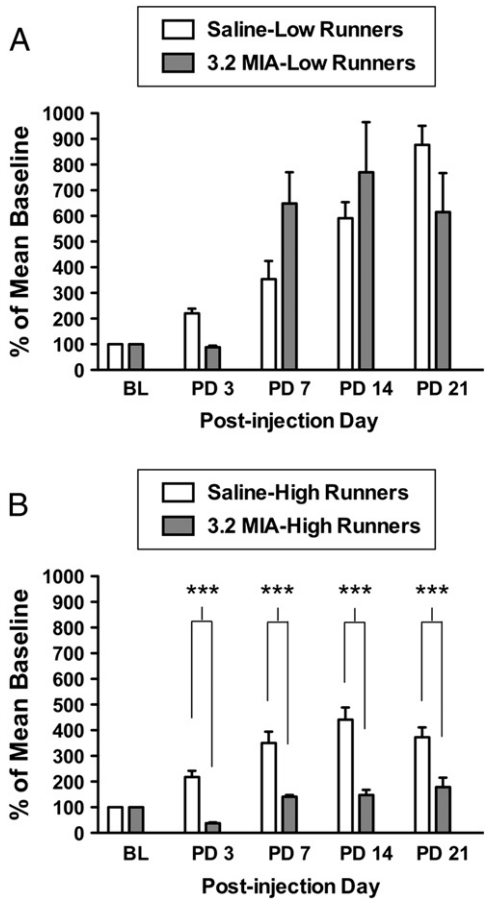

Fig. 6. % of mean (±SEM) baseline wheel running for low runners and high runners for Experiment II, with n = 4 per group. Panel A shows % of mean baseline running for low runners. Panel B shows % of mean baseline running for high runners. Abscissae: Post-injection day. Ordinates: % of mean baseline wheel running. For Panel B, Two-factor ANOVA showed a significant main effect for MIA concentration (saline vs. 3.2 mg), $F(1,4) = 30.37$, $p < 0.00$, Time (Day), $F(1,4) = 41.97$, $p > 0.0001$, and Interaction, $F(2,4) = 14.31$, $p < 0.001$. Post-hoc analyses revealed significant differences between saline and MIA runners at all post-injection days (***$p < 0.001$).

The first step in developing an assay of osteoarthritis-depressed behavior was to determine parameters that produced high levels of wheel running. For running wheel experiments, this was achieved by exposing rats to running wheels (acquisition phase) prior to administration of the chronic pain manipulation. Two different experiments were completed in which the duration of acquisition was varied. A preliminary study included a 42-day acquisition period (Stevenson et al., unpublished observations), however, the data were similar to that of Experiment I and thus the preliminary protocol was abandoned for the higher throughput Experiment I protocol. Experiment I included a 21-day acquisition phase whereas Experiment II included a 7-day acquisition phase. On the final acquisition days of Experiments I and II, rats were running approx. 10,500 m and 2500 m, respectively. Baseline running rates for Experiment I were high and approached stability by acquisition day 17, whereas baseline running rates for the higher throughput Experiment II steadily increased but never stabilized. The constantly increasing baseline in Experiment II may prove problematic for interpreting the ability of pain states to depress baseline running as well as the efficacy of analgesic drugs to restore pain-depressed running. A limitation of the Experiment II protocol may be that it has less predictive and/or face validity than protocols with stable baselines such as Experiment I. In an effort to further develop protocols that yield stable baseline behavior, our laboratory has begun to assess higher-throughput/short-duration experiments in which rats get 30–120 min access to wheels (see below).

The next step was to determine the ability of MIA to depress wheel running. There is a literature base on the ability of osteoarthritis to depress physical activity in humans and thus the determination of MIA's effects on wheel running may represent a clinically relevant endpoint (Quellet and Moffet, 2002; Van Gool et al, 2007; Zeni and

Higginson, 2009). MIA (3.2 mg) produced a brief and modest depression of wheel running in Experiment I. In marked contrast, MIA (3.2 mg) produced a long-lasting and robust depression of wheel running in Experiment II. Thus, MIA was relatively ineffective in depressing wheel running in rats that had a longer acquisition phase (21 days/Experiment I), whereas MIA was more effective in depressing wheel running in rats that had a shorter acquisition phase (7 days/Experiment II). It follows then, that the differential efficacy of MIA to depress wheel running may be a function of acquisition duration. In support of this and in contrast to other forms of locomotor activity (e.g., open field LMA), rats with voluntary access to running wheels exhibit steady elevation in running distance and/or rates across successive days, until a plateau is reached after several weeks of running (Latanzio and Eikelboom, 2003; Smith and Yancey, 2003). These data are consistent with the present findings that rats with 21 days acquisition (Experiment I) achieved very high rates of running and that running stabilized between days 17–21. In contrast, rats with only 7 days acquisition (Experiment II) demonstrated steadily increasing rates of running that were relatively low on the final day of acquisition (2500 m) compared to the final day of acquisition for rats in Experiment I (11,000 m). Thus, because running distance was comparatively low and still being acquired in Experiment II, it is possible that wheel running was more susceptible to suppression by MIA. Second, it is also possible that wheel running may structurally strengthen the hip, leg bones, knee and surrounding cartilage (De Jong et al., 2004; Kelley et al., 2000; Kemmler et al., 2004; Stevenson et al, unpublished observations), with the prediction that longer durations of running would be more protective of subsequent musculoskeletal pain manipulations, and therefore, longer durations of running acquisition (e.g., Experiment I) would be more difficult to depress with chronic pain. This idea is consistent with data showing that moderate and high intensity exercise can slow down age-related decreases in hip bone mineral density in patients with rheumatoid arthritis and co-morbid osteoporosis (De Jong et al., 2004), maintain or increase femur bone mineral density in men (Kelley et al., 2000), and reduce lumbar and femur bone loss and back pain in postmenopausal osteopenic women (Kemmler et al., 2004).

Of note was the finding that MIA-depressed running depended on baseline levels of wheel running prior to MIA administration. Specifically, MIA produced robust depression of running in rats with high baseline running rates, whereas MIA produced only sporadic and moderate depression of running in rats with low baseline rates. The reason for this finding is unknown. The apparent rate-dependent effects of MIA are reminiscent of several seminal studies by PB Dews, DE McMillan and others (Dews, 1955; Leander and McMillan, 1974; Wenger and Dews, 1976) who reported that the effects of drugs on behavior varied as a function of the underlying baseline rates of behavior. For example, the stimulant amphetamine and the generalized behavioral depressants pentobarbital, chlorpromazine and ketamine were shown to produce robust depression of relatively high rates of responding, and minimal depression or even stimulation of lower rates of responding within a Fixed-Interval schedule of reinforcement (Leander and McMillan, 1974; Wenger and Dews, 1976) in both pigeons and mice. Thus, just as baseline rates of schedule-controlled responding are powerful determinants of the effects of drugs on response rates (Dews, 1955), perhaps baseline rates of behavior are powerful determinants of the effects of subsequent pain states on that behavior. In addition, it has been widely reported that rates of running are highly variable in rats with 24 h access to running wheels (Smith and Yancey, 2003; Smith and Lyle, 2006), and these reports are consistent with our findings of variation in running during baseline acquisition and post-MIA observation phases. The differential ability of MIA to suppress running as a function of baseline rate speaks to the challenges of using a running wheel protocol to quantify nociceptive behaviors. Further, it may be the case that analgesics will be differentially effective at restoring pain-depressed running in low vs. high runners and clearly, baseline rates will need to be tracked in future studies that utilize wheel running as a dependent measure. An alternative approach would be to provide limited access to running wheels (e.g., 2 h/day) in an effort to stabilize baseline running patterns. Our laboratory has been exploring 30, 60 and 120 min daily access sessions and has found that rates of running stabilize much sooner than when rats have 24 h access (Stevenson et al., unpublished observations).

The effects of MIA were also determined in an assay of pain-stimulated behavior. In this test, MIA produced a concentration-dependent increase in tactile sensitivity (mechanical allodynia). These findings with the von Frey test are consistent with other reports demonstrating the ability of MIA to induce mechanical allodynia (Beyreuther et al, 2007; Combe et al, 2004; Fernihough et al, 2004). For example, 2 mg MIA produced time-dependent mechanical allodynia, with tactile hypersensitivity being most robust from MIA post-injection day 14 onward, and lasting up to 63 days (Combe et al, 2004; Fernihough et al, 2004). In the present study, MIA was more potent in producing mechanical allodynia than in depressing wheel running, as a dose of 1 mg MIA was effective in decreasing paw withdrawal thresholds, whereas a dose of 3.2 mg MIA was needed to depress wheel running. In addition, the time course of MIA was different between these two tests. Specifically, in the von Frey test, 1 and 3.2 mg MIA produced lasting tactile sensitivity at all days tested (post-injection days 3, 7, 14, and 21), whereas 3.2 mg MIA depressed running from days 1 to 4 and from days 10 to 21.

Additionally, the effects of MIA were determined in an assay of hind limb weight bearing. In this test, MIA produced a time- and concentration-dependent shift in hind limb weight bearing, onto the uninjured, contralateral side (a putative measure of spontaneous pain). These results with the weight bearing apparatus are also consistent with reports showing MIA's effects on posture in rats (Bove et al, 2003; Combe et al, 2004; Fernihough et al, 2004). In the present study, MIA was most potent in the weight-bearing test relative to the von Frey test and wheel running assay. In particular, a dose as low as 0.32 mg MIA produced a significant shift in weight bearing on post-injection day 3 whereas higher doses were necessary to produce mechanical allodynia (1 mg) or depress wheel running (3.2 mg).

Finally, the effects of MIA on articular cartilage and subchondral bone were determined using Hematoxylin and Eosin staining. MIA produced a concentration-dependent reduction in cartilage density and increase in subchondral bone remodeling. This profile of effects is consistent with MIA's mechanism of action, namely, disruption of chondrocyte glycolysis and eventual cell death (Bove, et al., 2003), and is similar to published reports showing MIA-induced damage to bone and cartilage. For example, several studies on OA disease progression demonstrate transient inflammation during post-injection days 1–4, followed by concentration-dependent loss of articular cartilage, subchondral bone sclerosis and osteophyte formation at joint margins from post-day 14 onward (Guingamp et al., 1997; Bove et al., 2003; Fernihough et al., 2004). The transient inflammation (post-days 1–4) followed by pathological changes to bone and cartilage reported in the literature, also tracks well with our running wheel data that demonstrate initial MIA-induced suppression of wheel running around day 3 followed by more robust suppression of running following day 14.

In summary, MIA was effective in producing pain-depressed wheel running. Given that MIA was less potent in producing depression of wheel running than tactile allodynia or shifts in weight bearing, one interpretation of these data might be that wheel running may be the least sensitive measure of MIA-induced osteoarthritis. In contrast, the finding that MIA produces apparent rate-dependent effects on wheel running may indicate the subtle complexities of characterizing the effects of chronic pain states on normally adaptive behaviors. It is becoming increasingly accepted that characterization of the validity of current animal pain models as well as development of relevant new

dependent measures is warranted (Martin et al., 2004; Mogil, 2009; Negus et al., 2006; Piesla et al., 2009; Roughan and Flecknell, 2000, 2003; Vierck et al., 2008). Toward that end, the present study sought to characterize the effects of chemically induced osteoarthritis on a battery of qualitatively different behaviors. Future studies that evaluate analgesic and non-analgesic drugs on the above battery of behaviors will be an important next step in assessing the predictive validity of pain-depressed behaviors, and may help in determining whether or not such measures have utility as part of a more comprehensive approach to the preclinical assessment of pain and analgesia.

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