Deficits in spontaneous burrowing behavior in the rat bilateral monosodium iodoacetate model of osteoarthritis: an objective measure of pain-related behavior and analgesic efficacy

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Objective: To characterize deficits in burrowing behavior — an ethologically-relevant rodent behavior — in the monosodium iodoacetate (MIA) rat model of osteoarthritis (OA), and the sensitivity of these deficits to reversal by analgesic drugs of both prototypical and novel mechanisms of action. A second objective was to compare the burrowing assay to a spontaneous locomotor activity (sLA) assay.  
Method: Male Wistar Han rats (200–220 g) received intrarticular (i.a.) injections of MIA or saline for sham animals. A deficit in the amount of sand burrowed from steel tubes filled with 2.5 kg of sand was used as a measure of pain-related behavior, and sensitivity to reversal of these deficits by analgesic drugs was assessed in bilaterally MIA-injected rats.  
Results: Bilateral MIA injections induced a significant impairment of burrowing behavior, which was concentration-dependent. The temporal pattern of the deficits was biphasic: a large deficit at 3 days post-injection, resolving by day 14 and returning at the 21 and 28 day time points. At the 3 day time point ibuprofen, celecoxib and an anti-nerve growth factor (NGF) monoclonal antibody (mAb) were able to significantly reinstate burrowing behavior, whereas the fatty acid amide hydrolase (FAAH) inhibitor PF-04457845 and morphine displayed no reversal effect. Morphine impaired burrowing behavior at 3 mg/kg in sham animals. Deficits in rearing frequency in the locomotor activity assay proved irreversible by analgesics.  
Conclusion: Burrowing behavior provides an objective, non-reflexive read-out for pain-related behavior in the MIA model that has predictive validity in detecting analgesic efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) and an anti-NGF mAb.

Introduction

The prevalence of osteoarthritis (OA) is estimated to be 50% in people aged 65 and older1 and with an aging population the cost and health impact of this disease will continue to rise. The intra-articular (i.a.) injection of monosodium iodoacetate (MIA) into the rat knee joint produces histological changes representative of those seen in human OA2. Concomitant with the early and late histological changes in the MIA model are pain-related behaviors3, making it a useful model for assessing the analgesic efficacy of drugs.

Pain is the main clinical manifestation of OA. The current lack of effective disease modifying agents to target the aetiology of OA4 means the use of analgesic drugs is the mainstay treatment for alleviating the impact of the disease on daily life, namely non-steroidal anti-inflammatory drugs (NSAIDs) and weak opioids5.

The main techniques for assessing pain-like behaviors in the MIA model are measures of mechanical hypersensitivity: shifts in weight bearing (WB) from the ipsilateral affected to contralateral unaffected knee, distal mechanical hyperalgesia and tactile allodynia6–8. A pressing issue in the pain field is the lack of translation between preclinical and clinical findings, which has resulted in relatively few safe and effective analgesics being developed (reviewed by Blackburn-Munro 20049 and Mogil, 200910).
Although there is evidence that current pain assays have some predictive validity from rat to human\textsuperscript{12}, it is becoming increasingly apparent that assays utilizing spontaneous rather than evoked/reflexive measures are needed to assess the global impact of pain beyond hypersensitivity\textsuperscript{22,23}.

In addition to sharp pain evoked by movement, patients with OA rank constant and aching pain among the most distressing features of the disease\textsuperscript{12}. How well the current evoked/reflexive assays of pain-like behaviors in rodents account for this persistent pain is unclear\textsuperscript{2}. Furthermore, clinical assessments of chronic pain are focusing increasingly on the multifaceted nature of pain, such as the effect on emotion and physical function\textsuperscript{15}, which are not assessed preclinically by reflexive assays.

Burrowing is an innate rodent behavior indicative of animal well-being that is conserved across various strains of rat\textsuperscript{16–19} and mice\textsuperscript{20–22}. Deficits in burrowing behavior occur in preclinical rat models of inflammatory and neuropathic pain and can be reversed by analgesics\textsuperscript{16,18,19,21,23,24}. This behavior, therefore, is a useful preclinical measure of non-evoked pain. Burrowing may encompass the supraspinal mechanisms that contribute to pain phenotypes, both sensory and affective, as well as being more ethologically relevant than the commonly used assays measuring hypersensitivity\textsuperscript{25}.

Here we show deficits in burrowing behavior in the bilateral MIA model of OA and sensitivity of these deficits to reversal by prototypical and novel analgesics. To our knowledge, this is the first published article demonstrating deficits in burrowing behavior in the MIA model. Additionally, burrowing behavior was compared to another non-reflexive readout: spontaneous locomotor activity (sLA). This has been shown previously to be impaired in various preclinical animal models and to be sensitive to pharmacological modulation\textsuperscript{26–29}.

Methods

Animals

594 male Wistar Han rats (200–220 g; Charles River Laboratories, Germany) were used for all experiments and were housed in groups of four with food and water ad libitum with a 12 h light/dark cycle. All animal experimental protocols were authorized by the Local Animal Care and Use Committee and carried out according to the local animal care guidelines, AAALAC regulations, and the USDA Animal Welfare Act.

MIA injections

Rats were briefly anaesthetized with 5% isoflurane (Abbott Laboratories, Wiesbaden, Germany) followed by 3% maintenance, after which an i.a. injection of 3 mg of MIA (Sigma–Aldrich, Steinheim, Germany) dissolved in 50 μL of 0.9% physiological saline was performed into the femorotibial joint. Sham rats received 50 μL injections of physiological saline. All injections for pharmacology experiments were bilateral, except for one model conditions studies in which rats received either uni- or bilateral injections.

Drugs and drug administration

The analgesic drugs tested were: morphine (Caelo, Germany), ibuprofen (Sigma Aldrich, Steinheim, Germany), celecoxib (LKT Laboratories Inc., St. Paul, MN, USA) and the fatty acid amide hydrolase (FAAH) inhibitor PF-04457845 (synthesized by Boehringer Ingelheim). All drugs were administered orally (p.o.) with 0.5% (w/v) natrosol and 0.1% (v/v) tween-80 (9:1 ratio) as vehicle, except morphine which was injected subcutaneously (s.c.) in the interscapular area with 0.9% physiological saline as vehicle. All drugs were administered in a volume of 2 mL/kg except PF-04457845, which was administered in a volume of 4 mL/kg.

For the generation of the anti-nerve growth factor (NGF), monoclonal antibody (mAb) variable domains were extracted from the patent application WO 2004/058184 A2 (applicant: Rinat Neuroscience Corporation) and processed as described by Hazereh et al., 2001\textsuperscript{30}. The antibody was administered s.c., with phosphate buffered saline as vehicle.

Burrowing training and burrowing experiments

For all burrowing experiments steel tubes (32 cm in length and 10 cm in diameter) were filled with 2.5 kg of quartz sand and placed in Plexiglas cages (600 × 340 × 200 mm). The open-end of the tube was elevated 6 cm from the floor of the cage. Training for each experiment was carried in 2 phases: social facilitation (SF) and individual training (IT). For SF rats were placed in pairs in a cage for 2 h on two consecutive days. The amount of sand burrowed by each pair was measured and if a pair burrowed less than 1500 g of sand one of the pair was swapped with a rat from a pair that had burrowed greater than 1500 g for the second SF day. For IT rats were placed alone in the burrowing set-up for 30 min per day and the average amount burrowed over 3 days was calculated to attain a baseline burrowing performance value.

Before each animal was given MIA or sham intrarticular injections they were assigned to groups to ensure that each treatment group for an experiment had a comparable baseline burrowing value. For the model conditions study rats were allowed to individually burrow for 30 min 3 days after MIA or sham injections were performed and the amount of sand burrowed was recorded. For the time-course experiment burrowing performance was assessed 3, 14, 21 and 28 days post-injection. For all pharmacology experiments burrowing behavior was measured 3 days after MIA injection, and animals were placed in the burrowing set-up after the appropriate pre-treatment time.

Exclusions

During training any animal that has a baseline burrowing value of less than 1000 g or a standard deviation of burrowing (SD) greater than 450 g was excluded to ensure high and stable baselines. To ensure that all rats used in pharmacology experiments were in a comparable pain state burrowing values were also measured 1 day after MIA injection and any animal with a burrowing value greater than 1000 g was excluded from pharmacology at day 3. In total, exclusions after training and after day 1 accounted for around 5% of rats per study.

Locomotor activity

sLA was measured for 30 min with an automated monitoring system (TruScan Activity Monitor version 2.0, Coulbourn Instruments, Allentown, PA, USA). Each monitoring system was an enclosed 43 cm\textsuperscript{2} arena with two levels of sensory photobeams; one level elevated 3 cm from the floor of the arena and the other 14 cm, measuring horizontal and vertical activity respectively. Each level of the detection system was equipped with 16 photobeams per wall of the arena, spaced 2.5 cm apart. The parameters measured by the system were: ambulatory horizontal distance moved (cm), rearing frequency and rearing time (s). Prior to testing, all rats were habituated in an annexe to the testing room.

Statistical analysis

All statistical processing was performed in GraphPad Prism version 6.0 (San Diego, CA, USA). For all analyses P < 0.05 was
considered statistically significant. To control for familywise type I error rates a Bonferroni correction was applied where appropriate. A 1-way analysis of variance (ANOVA) with Bonferroni’s post hoc was used to analyze the model conditions studies investigating whether uni- or bilateral MIA injections were most appropriate and the concentration-dependency of burrowing deficits. A Bonferroni cut-off of $P < 0.025$ was applied: 0.05/(number of comparisons per family (2)/number of families(1)). The time-course study was analyzed using 2-way repeated measures ANOVA. Significant differences between sham animals and MIA-injected animals on the same day were ascertained using Bonferroni’s post hoc. A Bonferroni cut-off of $P < 0.025$ was applied: 0.05/(number of comparisons per family (4)/number of families(2)). The statistical significance of deficits in locomotor activity parameters was analyzed using an unpaired Student’s t test.

For all pharmacology studies (burrowing and locomotor activity) data were analyzed using a 2-way ANOVA. To determine that the MIA vehicle group was significantly lower than the sham vehicle group and to determine which doses of compound were efficacious in reversing burrowing deficits a Dunnett’s post hoc multiple comparisons was used, comparing with the MIA vehicle control group. All data are expressed as means and corresponding 95% confidence intervals (CI). Gaussian distribution was analyzed using a Shapiro–Wilk normality test.

**Results**

*Establishment of model conditions*

To determine whether unilateral or bilateral MIA injections were most appropriate for the burrowing assay an experiment was conducted for comparison. Injection of MIA bilaterally resulted in a 60% reduction in burrowing performance compared with the sham bilateral control group 3 days post-injection, whereas unilaterally MIA-injected rats displayed no deficit in burrowing performance at day 3 [Fig. 1(A)], or at later time points of 14, 21 and 28 days [Fig. 1(C)]. We investigated the effect of saline sham injections on burrowing performance and found that neither unilateral nor bilateral injections of saline influenced burrowing behavior [Fig. 1(A)]. Next we wanted to establish the concentration-responsiveness of MIA-induced burrowing deficits. Concentrations of 0.3, 1 and 3 mg/knee were selected based on previous findings in WB6. The 0.3 mg/knee concentration of MIA did not significantly impair burrowing performance, whereas 1 and 3 mg/knee induced significant deficits [Fig. 1(B)]. Although 1 and 3 mg/knee concentrations produced comparable deficits, we decided on 3 mg/knee for pharmacological experiments as this concentration produced the most robust deficit with the least variance.

To establish time points for pharmacological experiments burrowing performance was assessed 3, 14, 21 and 28 days after the bilateral injection of MIA [Fig. 1(C)]. As seen in [Fig. 1(C)], a robust deficit occurred at 3 days post-injection, which resolved back to baseline values by the 14 day time point. However, the deficits at the 21 and 28 day time points in the pharmacology experiments were found not to be large enough to provide an assay window to titrate a reversal effect. Therefore, it was decided to focus on the 3 day time point for all pharmacology.

After establishing the model conditions for the burrowing assay, the same conditions were chosen for locomotor activity studies to allow for comparisons between these two assays. Three days after bilateral injection of MIA (3 mg/knee) deficits in distance moved [Fig. 2(A)], rearing frequency [Fig. 2(B)] and rearing time [Fig. 2(C)] occurred. We decided to use rearing frequency as the parameter for pharmacology studies as it had the largest deficit; rearing frequency was 48% lower in MIA injected animals than in sham animals.

**Effect of NSAIDs, morphine and gabapentin on deficits in burrowing performance**

Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are categories of drugs that are currently used to treat pain in OA.
clinically\textsuperscript{5}, so were assessed to determine their efficacy in reversing deficits in burrowing performance 3 days after the injection of MIA. Both the non-selective cyclooxygenase (COX) inhibitor ibuprofen and the COX-2 selective inhibitor celecoxib were efficacious in reinstating deficits in burrowing performance at doses of 10 and 30 mg/kg for ibuprofen, and 3, 10 and 30 mg/kg for celecoxib [Fig. 3(A) and (B), respectively]. The increase in burrowing performance elicited by ibuprofen and celecoxib at these doses was a minimum of 50\% when compared with the MIA vehicle group.

Neither morphine or gabapentin reversed burrowing deficits at any dose tested [Fig. 3(C) and (D)], and morphine impaired burrowing performance at 3 mg/kg in the sham group.

**Effect of an anti-NGF mAb and the FAAH inhibitor PF-04457845 on deficits in burrowing performance**

An anti-NGF mAb was tested in the burrowing in light of recent phase III clinical trials with an antibody of the same mechanism of action\textsuperscript{14}. When given 24 h before measurement of burrowing performance the antibody had no effect on sham animals and was able to reverse the deficit in burrowing behavior induced by MIA to a level similar to that of the respective sham group, at a dose of 9 mg/kg [Fig. 4(A)].

The FAAH inhibitor PF-04457845 is another analgesic with a novel mechanism of action tested in burrowing because of recent negative phase II clinical trials in patients with knee OA\textsuperscript{32}. At all doses tested (10, 30 and 100 mg/kg) there was no reversal of burrowing deficits observed in MIA injected rats and also no effect on burrowing performance in the sham animals [Fig. 4(B)].

**Locomotor activity pharmacology**

Supplementary Table 1 shows that in our hands no analgesic effect of any drugs tested was detectable in the rearing frequency parameter of the locomotor activity assay, which is in contrast to the pharmacological sensitivity evident in the burrowing assay. The 10 mg/kg dose of morphine caused a significant reduction in rearing frequency compared with the sham vehicle group; however, unlike for burrowing, there was no significant reduction in rearing frequency induced by the 3 mg/kg dose (P = 0.18) compared with sham vehicle.

**Discussion**

The initial aim of this study was to investigate whether burrowing was impaired by i.a. injection of MIA and could therefore provide a measure of pain-related behavior in this pre-clinical model of OA. Our model conditions studies showed that injecting MIA into only one knee joint was insufficient to cause depression of burrowing behavior, however, bilateral injection resulted in a robust deficit in performance. One possibility for this is that when MIA injections are given unilaterally compensation occurs through use of the three unaffected limbs to burrow.

In contrast, previous studies investigating the effect of intraplantar (i.pl) complete Freund’s adjuvant (CFA), i.a. CFA and nerve ligation models of neuropathic pain found unilateral injury to be sufficient to depress burrowing behavior\textsuperscript{16,18,19,24}. It could be inferred that the MIA model is less severe in its pain phenotype and effect on animal well-being, therefore bilateral injury is needed to induce a deficit. However, unilateral injection of MIA is sufficient to induce deficits in wheel-running\textsuperscript{35} and locomotor activity\textsuperscript{35} in rats, perhaps suggesting burrowing is less sensitive to MIA-induced joint pain than these assays. Depression of locomotor activity after unilateral MIA injection — shown by More et al. (2013)\textsuperscript{34} — is in contrast to studies in our laboratory where no deficit occurred at the early (day 3) or late (day 28) time points (data not shown). The temporal pattern of MIA-induced deficits in burrowing performance was biphasic, which is similar to reported findings in WB\textsuperscript{5} and biotelemetric assessment of mobility (BAM)\textsuperscript{37}.

It has been suggested from behavioral and histological findings that the early phase of the MIA model (day 3 in our study) is predominantly pain mediated by inflammation\textsuperscript{5,6}, whereas the later stages of the model represent pain of a different aetiology, perhaps neuropathic in nature\textsuperscript{35}. This is inferred from observed upregulation of nerve injury markers at the late phase time points\textsuperscript{20–22} and sensitivity to drugs used to treat neuropathic pain\textsuperscript{37}. However, it is important to point out that previous studies mentioned here used unilateral MIA injections, whereas we
injected bilaterally — differences in pathophysiology between unilateral and bilateral injections should therefore be borne in mind. Taking this into account we suggest that the early phase deficits in burrowing represent inflammatory pain, which resolves by day 14 and a return of deficits in the late phase could be chronic/neuropathic in nature. We tested the neuropathic pain drug gabapentin at day 3 but found no effect at any dose tested, which is supportive of primarily inflammatory pain in the early phase of the MIA model. Unfortunately, the assay window for pharmacology at day 21 and 28 was found to be insufficient, therefore it was not possible to use gabapentin to discern whether this late phase was neuropathic in nature. It was estimated that 20–25 rats per treatment group would be required to determine a pharmacological effect at day 28.

These characterization studies show that deficits in burrowing behavior can be used as an objective measure of pain-related behavior in the MIA model of OA. Burrowing is a self-rewarding behavior that is not appetite-driven and is indicative of animal behavior in the MIA model of OA. Burrowing is a self-rewarding behavior can be used as an objective measure of pain-related logical effect at day 28.

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To investigate whether MIA-induced deficits in burrowing performance were sensitive to reversal by analgesics we tested a range of compounds that work by mechanisms of action currently used to treat pain associated with OA clinically. Both the non-selective COX inhibitor ibuprofen and the COX-2 selective inhibitor celecoxib (375-fold selectivity for COX-2) were efficacious in reversing deficits in burrowing performance at low doses of 10 and 3 mg/kg, respectively. Similarly, previous published findings found that a relatively low dose of ibuprofen (30 mg/kg) was efficacious in reversing deficits in burrowing induced by i.pl CFA. Both our study and this previous work demonstrate that burrowing may have higher sensitivity to the analgesic effect of ibuprofen than evoked endpoints. In the carrageenan model of inflammatory pain the minimally effective dose (MED) of ibuprofen to reverse mechanical allodynia was 300 mg/kg and to reverse thermal hyperalgesia 1000 mg/kg, which is considerably higher than the MED in burrowing. However, such comparisons should be approached with caution due to the differences in the underlying neurophysiological mechanisms between pain models and the strain of rat used for experimentation.

Morphine did not significantly reverse deficits in burrowing performance at any dose tested, and 3 mg/kg caused a significant reduction in burrowing performance in sham animals, presumably due to sedative side effects. This is in contrast to a previous published study which showed morphine to be efficacious in reversing burrowing deficits induced by i.a. CFA at 1 and 3.16 mg/kg, without reduction in burrowing performance in naive rats. However, the route of administration used was intraperitoneal (i.p.) in this previous study, whereas in this study morphine was administered s.c. The sedative side effects of morphine at 3 mg/kg was reversible, therefore it was not efficient, therefore it was not efficacious in reversing de

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assessment of pain in preclinical models is not ascertainable at this stage. Parallel studies investigating both spontaneous and evoked endpoints in the MIA model would allow for more comprehensive comparisons.

In contrast to burrowing, no analgesic effect of any drug was observed in sLA. Previous pharmacological characterization of MIA-induced deficits in rearing frequency demonstrated an acute analgesic effect of analgesics such as celecoxib and morphine. In our hands, reversal of burrowing deficits provides a more sensitive read-out for analgesic drug effect, which is in line with the findings comparing i.a. CFA-induced burrowing deficits and locomotor activity. Furthermore, deficits in burrowing performance are independent of motor impairment, as measured by the locomotor activity assay; we found no correlation between burrowing behavior or any locomotor activity parameters 3 days after i.a. MIA injections (data not shown).

The rationale behind the development of nonreflexive and ethologically relevant behavioral paradigms is to improve translation of preclinical to clinical findings. To complement this, the underlying pathophysiology of the animal model used should be as close to the human condition as possible. In that regard, the late phase (28 day time point) of the MIA model is perhaps more translational as it is suggested to model the chronic, noninflammatory pain that is more relevant to OA when it presents clinically. However, it must be noted that denoting OA as ‘noninflammatory’ may refer to a subset of patients, as there is evidence for inflammatory markers in some cases. This highlights the complexity and heterogeneity of OA as a disease. Nevertheless, further studies are warranted to optimize the burrowing paradigm to enable pharmacological testing at the 28 day time point.

In conclusion, these data demonstrate that burrowing provides an objective read-out for pain in the early phase of the MIA model and is sensitive to reversal by NSAIDs and an anti-NGF mAb. As well as having the advantage of being objective, the lack of experimenter presence during the test period in the burrowing assay reduces the influence of stress on rodent behavior. This is illustrated by a recent study showing that rodent pain responses are inhibited by the presence of a male experimenter due to stress-induced by axillary secretions. We also found that burrowing has superior sensitivity to pharmacological modulation over sLA, which is in line with findings in the i.a. CFA model of rheumatoid arthritis, further supporting the utility of burrowing as a preclinical pain assay.

Fig. 4. The effect of the anti-NGF mAb and the FAAH inhibitor PF-04457845 on burrowing performance 3 days after bilateral 3 mg/knee MIA or sham injections. Data analyzed with 2-Way ANOVA followed by Dunnett’s post hoc multiple comparisons. (A) anti-NGF mAb administered 24 h before burrowing performance was measured (MIA × dose interaction: $F_{5,80} = 7.273$, $P < 0.05$; $###P < 0.001$ vs sham vehicle, **$P < 0.01$ vs MIA vehicle). $N = 8$ per group. (B) PF-04457845 (p.o.) administered 2 h before burrowing performance was measured (main effect of MIA: $F_{2,65} = 54.65$, ****$P < 0.0001$). $N = 8–10$ per group.

Contributions

HD and JN contributed by conception and design of the studies, interpretation of data and critical revision of the article. LB generated, analyzed and interpreted the data and drafted the article. AP contributed by conception and design of the studies, interpretation of data and writing the manuscript.

Role of the funding source

All funding was provided by Boehringer Ingelheim Pharma GmbH & Co KG.

Competing interests

All authors were all employees of Boehringer Ingelheim Pharma GmbH & Co KG at the time of data collection and manuscript writing.

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References

12. Mogil JS, Crager SE. What should we be measuring in behavioral studies of chronic pain in animals? Pain 2004;112:12–5.


