Characterization of Burrowing Deficits in the MIA Model of Osteoarthritis

L.A. Bryden, N. Gorodetskaya, J.R. Nicholson and A. Pekcec

Department of CNS Diseases Research, Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany

Burrowing deficits as a measure of pain-related behaviour

Currently the assessment of pain-related behaviours in preclinical animal models relies on readouts that assess pain evoked by an external stimulus. These assays measure spinally-mediated reflexes that may not encompass supraspinal modulation of pain signalling and a number of reviews have highlighted the apparent lack of translation of these evoked measurements to the clinical setting [1,2]. Burrowing is an innate and self-rewarding behaviour naturally displayed and pain-induced deficits are a sensitive indicator of a reduction in animal well-being. These deficits are a specific read-out for pain in preclinical inflammatory and neuropathic pain models and are sensitive to reversal by analgesics, which validate the utility of this behaviour as an addition to current assays [3,4]. From a practical perspective, measuring burrowing behaviour does not require the animal to be restrained, as in some currently used evoked assays, which reduces the impact of stress as a confounding variable. Additionally, the lack of experimenter input during testing means burrowing is an objective assay that eliminates any possible experimenter bias. The injection of monosodium iodoacetate (MIA) into the rat knee joint induces osteoarthritis (OA)-like lesions with associated pain-related behaviours [5]. The aim of our study was to evaluate burrowing deficits in this preclinical model of OA and to establish if these deficits were reversible by analgesics.

Protocol and methods

Male Wistar Han rats (7-8 weeks/200-220 g; Charles River, Germany) were used for all experiments. Steel burrows (32 cm in length and 10 cm in diameter with opening at the front entrance elevated 6 cm from the floor) were filled with 2.5 kg of quartz sand and placed in Plexiglas cages with the open end of the tube positioned so it faced a rear corner of the cage to avoid sand being kicked out of the cage.

The training protocol was in two main phases. The first phase is Social Facilitation (SF) in which rats are placed in the burrowing set-up in pairs on two consecutive days and the amount of sand burrowed is measured. If a pair burrows less than 1500 g of sand on the first day it is swapped with a pair which has burrowed greater than 1500 g. The purpose of the SF phase is to ensure the rats learn the behaviour and the natural tendency of rats to imitate each other's behaviour means that this phase strengthens the burrowing behaviour.

The second phase is individual training where rats are allowed to burrow for 30 minutes a day over three days and the amount of sand burrowed is averaged to get a baseline value for each animal. Any rat burrowing on average less than 1000 g of sand or has a standard deviation of greater than 450 g over the three days is excluded, which usually accounts for less than 2% of rats in a study. The rats are then assigned to treatment groups based on their baseline burrowing values so that each group has a comparable baseline burrowing average.

To induce an OA-like state in rats MIA dissolved in 0.9% physiological saline is injected into the knee joint through the patellar ligament. In our first characterization studies we compared burrowing deficits in rats injected unilaterally with MIA to those injected bilaterally with MIA 3 days after injection (3 mg/knee in 50 µL). Secondly, we investigated the concentration-responsiveness of the deficits at 3 days by injecting 0.3, 1 and 3 mg/knee bilaterally in three groups of rats. The final characterization experiment was to study the temporal pattern of these deficits at 3, 14, 21 and 28 day time points post MIA injection.

For pharmacology studies the efficacy of a range of analgesics in reversing deficits in burrowing behaviour were investigated 3 days after MIA injection. The drugs tested were: ibuprofen (3, 10 and 30 mg/kg, 2 hour pre-treatment time); celecoxib (3, 10 and 30 mg/kg, 2 hour pre-treatment time); morphine (0.3, 0.6, 1 and 3 mg/kg, 1 hour pre-treatment time); anti- nerve growth factor (NGF) monoclonal antibody (mAb) (9 mg/kg, 24 hour pre-

treatment time); the Fatty Acid Amide Hydrolase (FAAH) inhibitor PF-04457845 (10, 30 and 100 mg/kg, 2 hour pre-treatment time). Ibuprofen, celecoxib and PF-04457845 were administered periorally (p.o.) suspended in 0.5% Natrosol and 0.1% Tween-80 (9:1 ratio) as vehicle. Morphine and the anti-NGF mAb were administered subcutaneously (s.c.) with saline and phosphate buffered saline as vehicle, respectively. Additionally, in pharmacological studies the burrowing performance of rats was measured 1 day after MIA injection and any rat burrowing greater than 1000 g was excluded.

Results

The first experiments were to characterize burrowing deficits in the MIA model. We found that only bilateral MIA injections produced a large deficit (60% reduction in burrowing compared to sham group) in burrowing performance compared to sham injected animals (50 μL of 0.9% saline into each knee joint) and that 3 mg/knee was the concentration that produced the most robust and reliable deficits with the least variance (811 ± 122g). A time-course experiment showed a large deficit in burrowing performance at 3 days after injection, which resolved to baseline at day 14 and reappeared at 21 and 28 days after MIA injection. The 3 day time point was chosen for pharmacology as it produced a robust deficit large enough to provide an assay window for reversal by analgesics. It was found that burrowing deficits at the 3 day time point were reversible by ibuprofen (10 and 30 mg/kg), celecoxib (3, 10 and 30 mg/kg) and the anti-NGF mAb (9 mg/kg). Morphine did not significantly reverse burrowing deficits at any dose tested and 3 mg/kg caused a significant reduction in burrowing in sham animals, presumably due to the sedative side effects of this drug. The FAAH inhibitor PF-04457845 did not reinstate burrowing behaviour at any dose tested.

Conclusions

Pain induced by intra-articular injection of MIA impairs burrowing performance and this is reversible by analgesic compounds. This demonstrates that burrowing behaviour provides an additional read-out for preclinical assessment of pain behaviour in the MIA model of OA. Additionally, burrowing is sensitive to side effects of drugs such as morphine, which induces a deficit in burrowing performance at 3 mg/kg. This means that both analgesic efficacy and side effect profiling can be achieved in one assay, whereas currently side effects must be assessed via separate methods, such as the rotorod performance test.

Ethical Statement

All animal experimental protocols used for these experiments 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.

References

[1] Blackburn-Munro, G. (2004). Pain-like behaviours in animals – How human are they? *Trends in Pharmacological Sciences*, ***2,*** 299-305.

[2] Vierck, C.J., Hansson, P.T., Yezierski, R.P. (2008). Clinical and pre-clinical pain assessment: Are we measuring the same thing? *Pain, **135***, 7-10.

[3] Andrews, N., Legg, E., Lisak, D., Issop, D., Richardson, D., Harper, S., Huang, W., Burgess, G., Machin, I., Rice, A.S.C. (2012). Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *European Journal of Pain, **16***, 485-495.

[4] Rutten, K., Robens, A., Read, S.J., Christoph, T. (2014). Pharmacological validation of a refined burrowing paradigm for prediction of analgesic efficacy in a rat model of sub-chronic knee joint inflammation. *European Journal of Pain, **18***, 213-222.

[5] Pomonis, J.D., Boulet, J.M., Gottshall, S.L., Phillips, S., Sellers, R., Bunton, T., Walker, K. (2005). Development and pharmacological characterization of a rat model of osteoarthritis pain. *Pain, **114***, 339-346.