

# Burrowing as a Non-reflex Behavioral Readout for Analgesic Action in a Rat Model of Knee Joint Arthritis

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## Aim of investigation

Non-evoked readouts for assessment of analgesic efficacy against spontaneous pain have been proposed to improve the predictive validity of preclinical models for analgesia. As such, the development and validation of novel readouts which go beyond reflex-withdrawal assays and which rely more on innate animal behavior is necessary. Here we demonstrate that the normalization of innate rodent behavior suppressed by pain, such as burrowing, can be a useful alternative behavioral readout for assessment of analgesic efficacy. In a Complete Freund's Adjuvant (CFA)-induced model of knee-joint arthritis the effects of naproxen, ibuprofen and pregabalin were compared in a weight bearing, an open field and a burrowing assay.

## Methods

Male Sprague Dawley rats were injected with 150µl CFA (2mg/ml) into the knee, three days before testing in weight bearing, open field and burrowing assays. Naproxen (0, 50, 100 mg/kg, ip), ibuprofen (0, 30, 100 mg/kg s.c.) and pregabalin (0, 10, 30 mg/kg, i.p.) were administered 30, 90 or 60 minutes, respectively, before testing. Weight bearing on each hind leg was determined using a rat incapacitance tester, horizontal locomotor activity and rearings were recorded in an open field for 5 minutes and burrowing performance was measured by the amount of gravel left in a hollow tube after 30 minutes of presentation to the rat. All experiments were performed in accordance with company, national and international regulations and laws for animal care and welfare as well as the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983).

## Results

CFA-induced knee-joint inflammation caused marked reductions in weight bearing in the inflamed leg, open field activity and burrowing. Naproxen, ibuprofen and pregabalin were efficacious in normalizing weight bearing, but in the open field horizontal locomotor activity was not normalized by any treatment condition and rearing behavior was reinstated only by ibuprofen (100 mg/kg) and not by the other treatment conditions. On the other hand, naproxen (100 mg/kg), ibuprofen (31.6 mg/kg) and pregabalin (10 mg/kg) effectively reversed CFA-induced deficits in burrowing behavior.

## Conclusions

These experiments suggest that measuring burrowing performance is an alternative non-reflex readout relying on innate rodent behavior which is affected by pain and can be pharmacologically manipulated. The burrowing assay appears to be more sensitive to pharmacological treatment than locomotor activity assessments in an open field assay. Furthermore, as opposed to reflex withdrawal or weight bearing assays, burrowing has the benefit of dissociating selective analgesic doses of a drug from doses that induce locomotor impairment in the same animal.