INTRODUCTION

- Patients suffering from chronic pain often have trouble carrying out daily activities making this an important area for pain research
- Preclinical testing of analgesic efficacy has traditionally relied on reflex evoked assays that often yield false positive results
- There are minimal preclinical non-pain evoked assays that test for analgesic efficacy in rats
  - Burrowing is an innate behavior that is predicted to be suppressed by pain
    - Innate behaviors in rodents may better model humans carrying out their day-to-day activities
- Drug-induced normalization of burrowing after the development of pain may be a more clinically relevant readout for assessing efficacy of novel analgesic drugs

HYPOTHESIS & SPECIFIC AIMS

- We hypothesize that after inducing an acute or sub-acute pain state, burrowing in rats will be suppressed, and when treated with known analgesics, burrowing will be restored
- The specific aims of this study were to establish a pain-suppressed burrowing assay in our laboratory, assess and optimize key parameters, and begin assessing drug effects

METHODS

Subjects:
- Male Sprague-Dawley rats (Harlan, 150-175 g) were used for all studies
- Rats were housed two per cage in the University of New England Animal Care Facility under standard housing conditions with food and water ad libitum

Drug Treatments:
- Carprofen 5 mg/kg, subcutaneously (s.c.), given immediately prior to applicable burrowing session(s)

Burrowing:
- Rats were habituated to a burrowing box (161 cm x 191 cm x 111 cm) lined with either blue absorbent padding or no padding, which contained one elevated burrowing tube (32 cm x 10 cm) filled with 2500 g of aquarium gravel (experimental design shown in Figure 2)
- Rats were then assessed for their baseline measurement of burrowing behavior during a 60 min session within the burrowing box
  - Gravel remaining in the tube was weighed to calculate the amount burrowed (g)
- Two more burrowing sessions followed:
  1. After naive injection of analogical drug: carprofen (5 mg/kg, s.c.)
  2. After induction of a pain state (plantar incision surgery)

Plantar Incision Surgery:
- Rats were anesthetized with isoflurane (2.3%) via a nose cone for the entire procedure
  - A 1 cm long incision was made through the skin and fascia, the plantar muscle was then located and a 1 cm incision was made lengthwise in the muscle
- Hemostasis was reached and skin was closed by two mattress sutures of 5-0 nylon

EXPERIMENTAL DESIGN

**Figure 1.** Burrowing tube (left), burrowing boxes (middle), and fall set up (right) of equipment used for burrowing behavior tests

**Figure 2.** Timeline of experimental procedure

**Figure 3.** Assessment or burrowing behavior pre and post injury

**Figure 4.** Assessment of the effect that sex and flooring type has on altering the amount of gravel burrowed out of the tube

**Figure 5.** Combined data from three trials comparing burrowing behavior between flooring parameters

**Figure 6.** Comparison of burrowing behavior either during the animals light cycle (8-12PM) or their dark cycle (7-11PM)

**Figure 7.** Assessment of the effect that altering the test session length or the habituation regiment would have on burrowing levels

**Figure 8.** Assessment of novel burrowing parameters

RESULTS

Burrowing:
- Habituation and baseline testing showed rats have an innate behavior to burrow and do so in the model we have set up in the laboratory
- Figure 3 showed that the amount of gravel burrowed was significantly reduced following the development of post-surgical pain
- Burrowing behavior was restored with carprofen (5 mg/kg, s.c.)

Parameter Modifications:
- Blue pad flooring increased the amount of gravel displaced during burrowing sessions
- There was a trend for greater burrowing in male versus female rats

DISCUSSION

Burrowing:
- After habituation to the testing chamber, rats will burrow gravel out of the tube
  - Once a pain state develops (post-surgical pain) burrowing behavior is suppressed
    - These results allow us to potentially be able to use this assessment of burrowing as a novel approach to test analgesic drugs

Parameter Modification:
- Parameters were modified to further optimize the assay and we found that:
  - Blue pad flooring increases burrowing levels
  - Padded flooring may reduce the sound of falling gravel which may startle the rats
  - Animal noise had no observed effect on burrowing allowing for equal testing of male and female rats in this assay

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**FUTURE DIRECTIONS**

- Future studies will focus on optimizing techniques and parameters of the burrowing assay by using HDWebcams to assess post-surgery results with new analysis techniques
- To look at previously approved pain models, such as burn and neuropathic pain, to assess the affect on burrowing