Developing Burrowing as a Non-Evoked ReadOut Assessment for Use with Novel Analgesic Drugs

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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 a natural innate behavior that is predicted to be suppressed by pain
    - Inmate 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 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 (16 x 19 x 11 in) lined with either blue absorbent padding or no padding, which contained one elevated burrowing tube (32 cm x 10 cm) filled with 250g 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 analgesic 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 muscles 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

Figure 1. Burrowing tube (left), burrowing boxes (middle), and full setup (right) of equipment used for burrowing behavior tests

EXPERIMENTAL DESIGN

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

VIDEO ANALYSIS

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
- 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
- Conducting testing during either the rats light or night cycle caused no observed differences to burrowing levels
- Test session length was able to be shortened from 60-min to 15-min without observing a decrease in burrowing levels
- Habituation could not be shortened while keeping a consistent level of burrowing

Video Analysis:
- Results from the video analysis looking at entries into the tube, time spent in the tube, and time spent burrowing showed that a majority of activity took place in the initial 15 minutes

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
- If a compound was efficacious, burrowing behavior would be restored following injury (ideally the analgesic drug would not have an effect on burrowing in the non-pain state)

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 sex had no observed effect on burrowing allowing for equal testing of male and female rats in this assay
  - The optimal time of day to run this test appears to be during the first 2-hours of the animals light or dark cycle (8-10 AM or 7-9 PM, respectively)
  - The original test session length of 60 min can and has been shortened to 15-min, to aid in higher throughput on test days

Video Analysis:
- In order to further justify using 15 minute trials, and to look at new analysis techniques, 60 minute sessions were videotaped using HD webcams:
  - Time spent burrowing was analyzed, and the initial 15 minutes yielded the most burrowing activity
  - The most entries into the tube were seen within the initial 15 minutes
  - Time spent in the tube was the greatest within the first 15 minutes

FUTURE DIRECTIONS
- Future studies will focus on optimizing techniques and parameters of the burrowing assay by using HD Webcams 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

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