Monitoring Burrowing and Nest Building Behavior as Species-specific Indicators of Animal Wellbeing

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Impairment and pain in laboratory rodents

Legislative bodies and the general public both demand that suffering is minimized when animals are used for scientific purposes, which is important for both ethical and scientific reasons. In addition to refined experimental methods and good husbandry practice, the recognition of reduced wellbeing is a prerequisite to assure the welfare of laboratory animals. It is essential to be able to recognise and assess pain reliably so that it can be treated effectively, e.g. following painful experimental procedures or when animals are suffering from disease. Although the mouse is the most widely used laboratory animal species, there has been a lack of non-invasive methods to assess moderate, lasting pain.

As behavior can be observed easily in a non-invasive manner, and can provide meaningful indicators of animal wellbeing, behavioral changes have been proposed as a useful and simple means to assess welfare in small laboratory species [1, 2, 3, 4].

We conducted a series of experiments using two highly motivated, species-specific home cage behaviors, burrowing and nest building, as sensitive indicators of mild to moderate post-operative pain. These behaviors could also be used to test the efficacy of analgesic treatment. Mice of the commonly used C57BL/6J strain underwent a minor, one-sided laparotomy under inhalation anesthesia, with or without pain treatment (the non steroidal anti-inflammatory drug Carprofen). Control animals received anesthesia/analgesia only. We recognise that it is not usual practice to withhold perioperative analgesia, but the decision was made that it would be justified in this case because the aim was to achieve the benefit of better pain recognition for laboratory mice. All animal housing and experimental protocols were approved by the Cantonal Veterinary Department, Zurich, Switzerland.

Burrowing performance

This study investigated the potential use of burrowing performance as a measure of mild to moderate post-operative pain in laboratory mice. The influence of minor surgery on burrowing was analysed in C57BL/6J mice of both sexes, using a modified rodent burrowing test within the animal's home cage. A standard, opaque plastic water bottle filled with food pellets identical to those of the animal's normal diet was provided for burrowing, and an additional empty bottle was provided to serve as a shelter for the animal (see Figure 1). We measured the latency to the displacement of food pellets (burrowing) from the burrowing apparatus, using infrared sensitive cameras to record animals for 24 hours in the absence of a human observer.

Almost all (98%) healthy mice burrowed (mean latency 1.3 h). After minor surgery with anesthesia but no pain treatment, latency of burrowing was significantly prolonged (mean Δ latency 10 h). Analgesic treatment with Carprofen decreased the latency of burrowing after surgery (mean Δ latency 5.5 h) to the level found in mice that had only been anesthetized (mean Δ latency 5.4 h) or had received anesthesia and analgesia (mean Δ latency 4.6 h). Analgesia during surgery was associated with a significantly earlier onset of burrowing compared to surgery without pain treatment.

Nest complexity scoring

In a previous study we demonstrated a general correlation of post-operative pain and nest building performance in laboratory mice [2]. However, a standardised protocol for the assessment of pain using nest building

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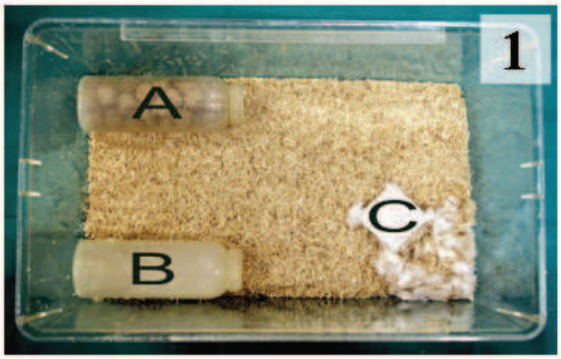

Figure 1. Experimental setup. A: Burrowing test apparatus, B: shelter, and C: nesting material (nestlet) in home cage [3].

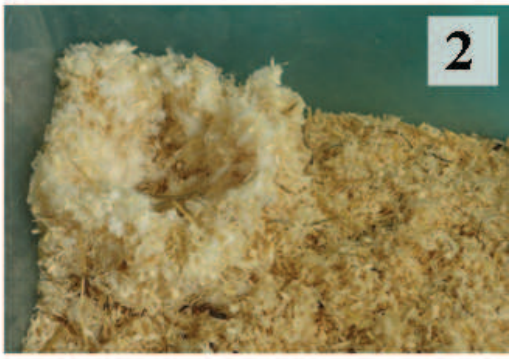

Figure 2. Example of complex nest: nest score 5; made from commercially available nestlet (Indulab).

performance has so far not been developed. Here we used a nest complexity rating scale to standardise nest scoring for the assessment of post-operative pain (score 0 = no nest building activity to score 5 = complex nest; see Figure 2) in female C57BL/6J mice. As mice tend to destroy and rebuild their nests in a circadian rhythm, successful assessment of nest building performance depends on the time when the nest is observed. Therefore we analysed the normal 24h nest building rhythm in healthy mice to determine a suitable time to score nest complexity.

Healthy mice spend 4.3% of their daily time budget on nest building and there are two peak times for this behavior; (i) in the beginning and middle of the light phase and (ii) in the second half of the dark phase. For pain assessment we choose a scoring time nine hours after the experiments, at the end of the light phase following the second nest building activity peak. Healthy mice had mean nest scores of 2-4 in baseline measurements. After experiments a significant decrease of nest complexity was seen with mean nest score 0 after surgery without pain treatment, 0.7 after surgery with pain treatment and 1.7 in the anesthesia only group.

Conclusion

Analyzing changes in species-specific behaviors has been suggested as a promising approach to assess both pain severity and the efficiency of pain management regimens [e.g. 3, 5]. The burrowing test and nest complexity scoring were both shown to be reliable and non-invasive tools for the assessment of post-operative impairment of general condition and pain in laboratory mice, and can be applied easily within the normal laboratory routine. However, detailed evidence for the efficacy of specific pain treatments will need further investigations.

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