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Effects of housing conditions on operant –conditioned rats

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The well-being of our animal research subjects is a primary concern of laboratory animal specialists. In the recent *Guide for the Care and Use of Laboratory Animals*, the housing of rodents in wire-bottom cages is discouraged due to its presumed effect on the rodents well-being. Housing in wire-bottom cages deprives the animals of their natural burrowing and nesting behaviors and may result in foot lesions. However, for many behavioral studies involving appetitive reinforcement, the use of wire-bottom caging is considered a necessity. Training and testing of rodents in food-or water-reinforced tasks necessitates moderate food or water deprivation to enhance the animal's motivation to learn and perform the task. For conditioning studies in which food is used as a reward, eliminating coprophagy by the use of wire bottom cages is considered to be a critical housing requirement. Unfortunately, there is little scientific documentation of the effects of wire bottom caging on the speed and accuracy with which animals acquire and perform in such tasks. In order to convince behavioral researchers that the use of wire-bottom caging is not a necessary component of their research, one must provide scientific evidence to back up the claim that housing in direct bedding cages does not affect behavioral performance and the related motivation processes. Our study was designed to test the hypothesis that there is no difference in the rate of task acquisition and asymptotic performance between rats housed in wire-bottom versus direct bedding caging.

Male BNxF344 rats (n=32), 4 months of age were housed in ventilated cages with either a wire bottom insert or directly on corncob bedding. The rats were trained according to an operant conditioning paradigm requiring them to maintain visual signal detection and discrimination performance over prolonged periods of time. This sustained attention task consisted of four shaping steps, each of which was mastered before the rat proceeded to the next step. In order to motivate the rats to perform in the operant chambers, they were 90% food deprived. The training study lasted six months. Analysis of data compared time to reach criterion performance for each of the 4 steps between the two housing conditions. Based on this analysis, there was no significant difference in task acquisition and asymptotic performance between the two housing conditions. Further study is being done comparing cortisol levels between the two groups of rats and their cortisol responses to acute and chronic stressors.

Based on the results of this study, rats do not require wire-bottom caging in order to maintain their motivation to perform in an operant conditioning task.

Telemetric measurements of effects of post-operative analgesics

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Objective

The Guide for the Care and Use of Laboratory Animals states, “unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals”. The implication from this statement is that any animal undergoing surgery (a painful procedure) must be provided with anesthetics, and more important in this application, post-operative analgesia. An essential aspect of a quality animal care program is the provision for post-operative analgesia unless scientific justification can be provided to indicate why this should not be done. The recommendation however comes with little scientific data on post-operative pain in rodents. We do not refute the concept that painful stimuli in humans are also likely to be present in animals. However, we believe the recommendation should come with scientific basis, not as a result of the lack of information as the statement in *The Guide* implies.

The goal of this study was to determine whether administration of preemptive and post-operative analgesia improves the well being of the mouse. We examined the effects of flunixin meglumine and buprenorphine in reducing pain and distress in adult female ICR mice that underwent a common surgical procedure (laparotomy). To test the efficacy of these agents, post-operative monitoring with telemetry was performed. Two hypotheses were tested in this experiment. The first study analyzed whether pre-emptive administration of analgesia improves analgesic activity over post-operative administration of analgesics. To examine this we tested the hypothesis that a single dose of flunixin meglumine (2.5mg/kg) or buprenorphine (2mg/kg) given prior to surgery would provide better post-operative analgesia than a dose given post-operatively. The second study examined whether multiple doses of buprenorphine improved analgesia compared with a single dose. Buprenorphine provides analgesia in the tail flick test for only 3-5 hours in mice. Thus, if post-operative pain continued beyond 5 hours additional dosages would be necessary for pain relief. We hypothesized that multiple dosages of buprenorphine given at 6 hour intervals would provide prolonged and improved post-operative analgesia compared with a single dose of buprenorphine.

Experimental Procedure

To test these hypotheses we implanted TA11PA-C20 radio transmitters (DSI) in the carotid artery of adult 35-45 gram female ICR mice. Following stabilization of the biologic parameters after the telemetry implantation surgery (at least 10 days) a mock ova implant surgery was performed. This involved a lateral flank laparotomy through a 1 cm incision and retraction of the ovary. The ovary was replaced and the skin sutured closed. We analyzed 6 mice per group. All data was collected for 10 seconds at 5 minute intervals beginning 1-2 days prior to surgery and extending at least 4 days after surgery.

Activity (ACT), heart rate (HR) and blood pressure (BP) were assessed by telemetry. We examined differences in the first 6 hours, and also as daily averages over the 4 day period post-surgery. Evidence of prevention of pain and distress was indicated when base line parameters were maintained for blood pressure, heart rate, and locomotor

activity levels, or if the changes were significantly less with analgesic administration compared with no analgesia.

Summary

Based on the preliminary data analyzed in several different ways, there was no evidence that a mock ova surgery caused significant prolonged pain in mice. Mild increases were seen in HR and BP for the no analgesia group; however these changes were not significant and were observed in most other treatment groups. Because of the absence of a significant effect from the surgery itself, interpretation of our 2 hypotheses is complicated. In the first hypothesis we proposed to test whether a single dose of flunixin meglumine or buprenorphine given prior to surgery would provide better post-operative analgesia than a dose given post-operatively. Only one condition was affected by pre-emptive analgesia. That was a decrease in HR when Banamine was given before surgery. Thus, the hypothesis that pre-emptive analgesia would be beneficial was not substantiated by this research.

The second hypothesis examined whether multiple dosages of buprenorphine given at 6 hour intervals would provide prolonged and improved post-operative analgesia compared with a single dose of buprenorphine. We found no benefit to administration of 3 doses of buprenorphine. In fact, the administration of multiple doses of buprenorphine did not improve the well being of the mice, and because of decreased food consumption and weight loss may have been more detrimental to the mice. In our observations, by 6 hours post-operatively any of the mild deviations that were observed began to return to normal. Thus, the additional administration of buprenorphine likely only led to further distress to the mice due to the extra handling.

Because of the equivocal results in the induction of pain in these studies, it is clear that further experiments are required. Determination of whether pain causes increases in BP and HR in mice like other mammals is necessary. More invasive surgical procedures may also warrant different analgesic agents. Finally, note that we used high doses of buprenorphine to assure effectiveness of analgesia. A lower dose may have fewer side effects. Please see the published manuscript: Goecke JC, Awad H, Caldwell-Lawson J, Boivin GP. Evaluating postoperative analgesics in mice using telemetry. *Comp Med.* 55:27-34, 2005 for further information.

EFFICACY AND SAFETY OF NEWLY PREPARED AND STORED TRIBROMOETHANOL IN ICR MICE

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This study, performed in conjunction with an *in vitro* evaluation of tribromoethanol (TBE), consisted of three trials with three objectives. The first objective was to compare anesthetic efficacy and short-term pathologic findings of TBE, ketamine/xylazine (K/X) and sodium pentobarbital (NaP). The second objective was to evaluate how changes that occur during the perceived most favorable and least favorable storage conditions [8 weeks at 5°C in the dark (5D) and 25°C with exposure to light (25L), respectively] affect anesthetic efficacy and short-term pathology when compared to a newly prepared TBE. The third objective was to perform a 6-week clinical assessment of animals that received newly prepared TBE.

For the first trial, animals that received TBE (400mg/kg), and 14 out of 15 that received K/X (120mg/kg/ 16mg/kg) were anesthetized, as defined by loss of pedal reflex. In comparison, only 8 out of 15 animals administered NaP (60mg/kg) were anesthetized. Anesthetic duration for animals that received K/X was 31.7 minutes, which was significantly longer ($p < 0.0085$) than animals that received TBE (18.5 minutes). Recovery times for TBE and K/X were not significantly different (26.5 and 27.5 minutes, respectively). Pathologic lesions associated with K/X administration were significantly less ($p < 0.0001$) than those associated with TBE. NaP was not associated with any pathologic lesions.

For the second trial, the pH of newly prepared and 5D TBE was 6.5-7.0, whereas the pH for 25L TBE was 3. Anesthetic induction, duration, recovery times and pathologic lesions were not significantly different, regardless of the pH or storage condition of the solution. It was noted however, that the average anesthetic duration for animals administered newly prepared TBE in the second trial was longer (37.7 minutes) than the first trial that used newly prepared TBE.

For the third trial (long-term clinical assessment), the average anesthetic duration for TBE was 46.5 minutes, significantly longer when compared to the two previous trials that used newly prepared TBE ($p < 0.025$). During this trial, 10 animals were found dead or found moribund and sacrificed. At necropsy all animals that were found ill exhibited a marked ileus.

In conclusion, TBE cannot be recommended due to variability in anesthetic effectiveness, pathology, and morbidity and mortality, regardless of the storage method. As a result of these findings, we do not recommend the use of Tribromoethanol in female ICR mice.

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CONTROLLED RELEASE OF MORPHINE IN MICE

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INTRODUCTION

Rodents, especially mice, represent the majority of laboratory animals used in biomedical research. At our institution, greater than 50% of mice used for research are assigned to studies that anesthetics or analgesics are required. Although the benefit of certain analgesic drugs is well demonstrated in mice, critics contend that the stress of repeated handling and dosing (oral or parenteral) of drugs is in itself distressful as well. Oral administration of analgesics via the drinking water or food is inadequate due to poor intake from discomfort, illness, or palatability.

Morphine is the standard opioid analgesic to which many analgesics are compared. It has documented efficacy and acceptance as an appropriate analgesic for mice. However, its short duration of action limits the widely employed use. Sustained release dosage forms are generally unavailable and have not been tested in laboratory animals appreciably. Therefore, morphine is a good candidate for incorporation into our biodegradable injectable gel system for controlled release. Therefore, the objectives of this study were to:

- (a) Investigate the effect of formulation factors on the *in vitro* release of a model opioid analgesic, morphine from biodegradable injectable gels and
- (b) Evaluate the *in vivo* performance of the drug-loaded gel formulations.

RESULTS

Both the specific objectives were fulfilled in this study and the results are reported in detail in the attached report. Specifically, preformulation studies were performed to determine the solubility and stability of the morphine base in the plasticizers used to prepare the gels. The particle size distribution of the drug used for the study was also measured. Following the preformulation studies, 12 formulations were tested for the *in vitro* drug release studies to evaluate the effects of varying formulation factors such as drug loading, polymer concentration, and hydrophilicity/hydrophobicity of plasticizers. The results of the preformulation study showed that the drug was stable in triethyl citrate (TEC) and acetyl triethyl citrate (ATEC). The solubility of the drug was 1 mg/mL in phosphate buffered saline (dissolution medium for the *in vitro* release studies), 0.64 mg/mL in TEC and practically insoluble in ATEC. An evaluation of the effect of varying formulation factors on *in vitro* release studies revealed that increasing the drug loading and hydrophilicity of the plasticizer increased the drug release. However, increasing the polymer concentration did not significantly decrease the *in vitro* drug release. Five different formulations were identified for evaluation of analgesia in ICR mice after examining the results of the *in vitro* release data. The formulations varied in polymer concentration, drug loading and injection volume. The results of the study showed that a formulation with 15% drug loading and 10% polymer was able to maintain analgesia in the animals for at least 5 days after a single subcutaneous injection of drug-loaded gels. The control formulation (blank gel without the drug) did not show any analgesia. The

injection site was resected to observe the gel residue at the site of injection after 4 hours and 6 days following the injection. A small lump of the gel residue was clearly visible at the site of injection 4 hours after injection, however, no gel residue was observed after 6 days post injection. Therefore, this study demonstrated that it is possible to maintain analgesia for at least 5 days following a single subcutaneous injection of drug-loaded gel in mice.

Determination of the Optimal Level of Vitamin E for the Treatment of Ulcerative Dermatitis in C57BL/6 and Related Strains of Mice

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Vitamin E has recently been shown to have a positive therapeutic effect on the skin lesions of ulcerative dermatitis (UD) of C57BL/6 and genetically altered mice with C57BL/6 background. However, the dose of vitamin E required to produce this positive effect is not known. In this study, we fed the standard NIH-31 control diet and the standard control diet with various levels of added vitamin E (1600 IU, 3300 IU, and 5000 IU per Kg of diet) to groups of 25 C57BL/6 and related strains of mice with spontaneous UD. The purpose was to determine the minimal amount of vitamin E required to achieve a therapeutic effect. Mice were randomly placed into one of the 4 diet groups as spontaneous cases of UD were identified within the vivarium. Mice were placed on diet regardless of age, sex, coat color or the site or number of UD lesions and sex, coat color, lesion location and the number of lesions on the body were recorded the first day. Lesion surface area in cm² was measured the day diet was initiated then weekly for 6 consecutive weeks. Statistically, all levels of vitamin E used in this diet study had a positive effect on lesion repair ($P < 0.05$). However, depending on the stringency of the parameters used to evaluate the differences, 100% or complete lesion repair versus 50% or better lesion repair, some diets performed better than others. In terms of complete lesion repair, 3300 IU per Kg diet of vitamin E was preferred, but when examined for 50% or better lesion repair, diets with 1600 IU per Kg diet vitamin E performed best. Therapeutically, the lower volume would be recommended since the responses to the diets are similar statistically. Sex, coat color, and the size of lesion at the onset of treatment did not influence repair, however, there was a moderate negative effect with lesion number. Because vitamin E is an antioxidant and its effects may alter study results, treatment at this time should be restricted to breeding animals and animals that have not entered research protocols. Future directions would be to determine the effects of a low dose of vitamin E on the long term feeding and prevention of lesion development.