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**Original Article**

# Buprenorphine Analgesia in Mice

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ABSTRACT

**Background:** Can buprenorphine analgesia for surgically-treated laboratory mice confound behavioral studies? **Objective:** Determine whether evidence to investigate this question was available from a retrospective analysis of data from Target Animal Safety (TAS) studies of an extended-release opiate analgesic in 32 male and 32 female BALB/cAnNCrl mice. Although the protocol-driven studies were designed to measure the toxicity of a new drug, regulatory guidelines required comprehensive clinical observations to monitor unexpected events including signs of paradoxical pain and stress. **Methods:** Studies used 5-fold excess doses of a buprenorphine suspension, 16.25mg/kg, in single and dose-repeat trials. Outcome measurements included body and organ weights, clinical laboratory, and histopathology analyses. Data were gathered twice daily to assess behavior and physical appearance across independent variables including external appearance, clinical signs, unprovoked behavior, and responses to external stimuli. The TAS studies demonstrated the safety of two successive treatments with this dose in male and female mice. **Results:** Mice treated with 16.25 mg/kg dose had increased eyes closed and decreased exploratory behaviors. **Discusion:** These results demonstrate that opiate therapy could confound behavioral studiess in surgically-treated mice in the early post-operative recovery period. Yet, while these transient behaviors could be taken as signs of stress, given the absence of remarkable differences in other parameters, they are interpreted as indicators of lethargy or drowsiness: side effects of opiate analgesia.

**Keywords**: Buprenorphine, Analgesia, Mice, Behavioral Observations.

## 1. INTRODUCTION

Opiate therapy is frequently used to meet standards for analgesia in research animals.[1,2] Opiates are front-line drugs for acute pain management in humans. The use of opiates for pain therapy in laboratory animals closely corresponds to the US Department of Agriculture Animal and Plant Health Inspection Service requirements for animal welfare, and principles for harmonizing laboratory models with

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pharmacology for human diseases.[3,4] Buprenorphine, a morphine analog, has a history of safe use in veterinary medicine and appears to be appropriate for pain management in laboratory animals, even when used in high doses.[5-12] Nonetheless, there have been questions about the effects of the multiple injections needed to provide 2-3 days of buprenorphine coverage, and whether the side effects associated with opiate therapy such as weight loss and nausea, may delay recovery of treated animals.[13-16] These questions have been addressed by research confirming that postsurgical weight loss associated with acute buprenorphine therapy is brief and seen only during the analgesic treatment period.[17,18] Recent studies have examined weight loss in mice and rats treated with long-acting buprenorphine preparations. Clinically significant weight loss in mice and rats has not been reported in these investigations.[19-22]

Questions remain regarding the behavioral effects of opiate therapy. Reports of opiate analgesia having both sedation and hyper-locomotor effects in rodents illustrate the complex interactions of drugs binding to opioid receptors.[19, 23-25] Investigator questions may influence decisions regarding the use of opiate, non-steroidal anti-inflammatory agents, or in special cases, administering no analgesia following potentially painful procedures. The concerns may be particularly relevant to behavioral studies with transgenic models of depression and neurodegenerative diseases where behavioral changes can be a marker for new genotypes. It is reasonable to investigate whether opioid-induced behavioral changes could obscure observation protocols that are standard tools for monitoring surgically treated mice.

We conducted Target Animal Safety (TAS) studies in surgically-treated mice to investigate the safety and efficacy of a lipid-bound buprenorphine analgesic.[22] Guidelines from the US Food and Drug Administration (FDA) for TAS investigations require tests using excess doses of the drug, and physical examinations during the studies.[26] The TAS protocol-driven examinations incorporated twice daily observations including records of physical signs and behaviors that are consensus indicators of pain and stress. In addition to observations focused on the surgical site, our studies collected approximately 20,000 data points regarding the behavior of the mice treated with drug and their drug-negative controls. The present report describes a retrospective analysis of the 480 observational records from mice in two trials that received a high dose of buprenorphine (16.25 mg/kg) or the negative control suspension for up to 4 days. We believe it is the first report correlating behavioral changes with the use of extended-release buprenorphine analgesia in mice.

## 2. MATERIALS AND METHODS

**Animals:**

Studies were approved by a University Institutional Animal Care and Use Committee (IACUC). The IACUC protocol complies with the National Research Council's Guide for the Care and Use of Laboratory Animals and fulfills the requirements of the Association for the Assessment and Accreditation of Laboratory Animal Care, International Program. The study was conducted at a University Department of Molecular and Comparative Pathobiology. Health surveillance and detection of pathogen contaminants were conducted by a soiled-bedding sentinel system. Male and female BALB/cAnNCrl mice (6-8 weeks old; weighing 20-22 g) were obtained from Charles River Laboratories (Wilmington, MA). Mice were inspected for general health conditions before being housed at a population of 4-5 mice per cage in Smart Bio-Pak cages (Allentown, NJ) with Tek-Fresh bedding (Harlan, Madison, WI), and were allowed free access to Teklad Global Rodent Diet chow (Harlan, Madison, WI) and defined water. Mice were held for approximately 10 days prior to the start of the experiment. Mice were weighed prior to assignment to the drug or control groups to ensure the weight of each mouse was within 10% of the average group weight at the start of the study. Throughout the duration of the study, mice were housed individually. Cages were changed daily to prevent buprenorphine re-dosing by coprophagy.

**Study Design:**

The study design was based on United States and European guidelines for assessing the safety of veterinary pharmaceutical products.[27,28] Because a previous study demonstrated the safety and unremarkable behavioral changes of 1, 3, and 5-fold doses of a powdered form of the drug, a 5-fold excess dose was utilized to examine safety and behavioral changes in this study.[29] The elimination period for buprenorphine in the extended-release drug was 3 days. Therefore, the study period was 4 days, 1 day more than the 3 day drug elimination period. Eight male and eight female mice per group were used in Trial 1 comparing a control (0.0 mg/kg) and 5-fold dose (16.25 mg/kg) challenge. Eight male and eight female mice per group were used in Trial 2 comparing control and 5-fold doses, repeated at three 4 day intervals. As shown in Table 1, at the midpoints of both trials, either Day 2 of Trial 1 or Day 6 of Trial 2, half of the mice (4 of each group) were weighed, euthanized, and exsanguinated for hematology and clinical chemistry analyses. At the endpoints of Trials 1 and 2, Day 4 and Day 12, respectively, the remaining mice were euthanized to measure body weight, hematology, clinical chemistries, and anatomic pathology. Mice were euthanized by carbon dioxide asphyxiation, exsanguination by cardiac puncture, and a thoracotomy. Body weights, hematology, clinical chemistry, gross pathology, and histopathology parameters evaluated in Trials 1 and 2 have been previously reported.[22]

**Drug and Control Preparations:**

The cholesterol-buprenorphine drug powder was supplied by Animalgesic Laboratories Inc. (Millersville MD). The drug powder contained USP (United States Pharmacopeia) grade buprenorphine HCl (Noramco, Wilmington DE), cholesterol, and glycerol tristearate, (Sigma, St Louis MO). Drug

preparations were verified for purity and content by AAI Pharma (Wilmington NC). Negative control, drug-free powder was prepared by tumble blending a mixture of cholesterol and glycerol tristearate (96/4, w/w) for 48 hours at 5°C.

**Drug Delivery:**

Injectable suspensions of drug powder and the control were prepared by suspending 80 mg of powder per mL of medium chain triglyceride (MCT) oil (Miglyol 812, from Sasol, Hamburg Germany) followed by brief shaking to make a homogeneous suspension. Suspensions were generally prepared within 1-2 days of use and stored at 2-8°C. A single (1x) dose consisted of a 0.05 mL drug suspension. One mL syringes with 1 inch 20 gauge needles were used to inject suspensions (described below) of cholesterol-triglyceride-buprenorphine powder and the negative control, cholesterol-triglyceride control powder.

**Surgical Procedure:**

The surgical procedure was based on the procedure used to implant Alzet mini-osmotic pumps in mice and rats. A video of the subcutaneous implantation procedure, which is briefly described below, is available at the Alzet website.[30] Mice were given intraperitoneal (IP) anesthesia with a solution containing 25 mg/mL ketamine, 2.5 mg/mL xylazine and 14.25% ethanol in saline. The dose of anesthesia was 0.15 mL/20 g mouse. When anesthesia was established, approximately 1 $cm^2$ of mid dorsal skin was shaved, washed with ethanol, and then coated with Betadine. Mice were transferred to a procedural table that was cleaned with 70% ethanol solution and covered with a clean disposable towel. A sterile disposable no. 10 blade was used to make a 4-5 mm incision through the skin only. Bleeding, if any, was controlled with sterile gauze and light pressure. Sterile forceps were used to separate the skin and to create approximately a 2 x 4 cm subcutaneous pocket. The skin was then apposed and stapled with 9 mm Autoclips (Kent Scientific, Torrington CT). After the skin was stapled, mice were injected with either the drug or control suspension (0.25 mL/mouse) into the interscapular subcutis. All mice in the study were treated to this "sham surgical procedure."

After the procedure, mice were moved to a holding cage. This cage contained a 37°C heating pad covered with a clean disposable towel. When the mouse regained consciousness, as demonstrated by movement and the absence of signs of distress, which included, but were not limited to, abnormal paw movements, efforts to scratch the incision site, and cowering, each mouse was placed individually into a clean cage.

**Clinical Observations:**

Two trials were performed for this study. Data per mouse were collected twice daily: in the morning, between 8-9 am, and evenings between 5-6 pm. The cage-side observations were conducted and recorded by the same female veterinarian, blind to the treatment groups. To quantify this data, FDA validated observation forms were used. The forms were designed for the entry of numerical grading of the extent to which pain or distress was present across nine parameters: mouse respiration, nasal/skin appearance, fur appearance, motor activity, ocular activity (closed or open eyes), behavior suggesting stress (i.e., aggressiveness), presence of tremors, and surgical site erythema, edema, or infection. Ratings were made on scales ranging from 1-3 or 1-6, depending on the parameter. Higher numbers indicated more severe signs of pain/distress. The ocular score was recorded using a scale of 1 to 5: 1, no observed abnormalities (NOA); 2, squinting; 3, eyes closed; 4, crusty secretions; and 5, porphyrin stain. The motor activity was recorded using a scale from 1 to 4: 1, NOA; 2, rapid darting; 3, hunched/lethargic, and 4, hunched/motionless. In addition, a "yes/no" score was given for an assessment of the general condition of each mouse and its cage. Space on the forms was also available for comment. Observations of the following were recorded twice daily: motor activity, ocular signs, fur appearance, and general conditions, (Table 2). The combined daily observation forms for each mouse hereinafter are referred to as the mouse chart.

**Statistics:**

Outcome of the experiments on ocular and motor activities were described as ordinal scores. Data were summarized as frequency or percentage. The possible natural variability of the ocular score and motor activity with respect to trial, gender, time (morning vs. afternoon), and day were assessed using data from the control group. Chi-square test was used for group comparisons and treatment comparison between the drug and the control. Subgroup comparisons were performed due to statistically significant confounding factors which were identified using the control data. All *P* values were reported as 2-sided, and all analyses were conducted using SAS software, version 9.2 (SAS Institute, Cary, NC).

## 3. RESULTS

Data collected from both trials showed that wound healing across groups was normal and comparable. There were no significant signs of bleeding, erythema, or edema at the surgical sites. With the exception of the motor and ocular scores, discussed below, there were no significant differences for the other behavioral parameters that were examined. For example, respiratory scores in both trials were similar for all mice. There were no signs of open-mouthed breathing or pronounced chest movement; no retching was observed. In the drug and control groups in both trials, several mice displayed labored breathing at times, but not consistently. Tremors were not seen in either trial. Nasal findings were negative; all mice received nasal scores of 1 indicating no crusty secretions or porphyrin stains. Aggressive behavior was not observed. There was no difference in the amount of abnormal paw movement, sluggishness, and cowering behavior exhibited by drug treated and control mice. Surgical site scratching was absent in both trials. Fur appearance was not affected by treatment

group or trial. Scores of 3, denoting a soiled or dirty coat, were common in both drug treated and control mice, likely due to the oily residue surrounding the injection site. Otherwise, fur and skin appeared normal. In the male arm of the repeat-overdose trial, 2 of 8 mice died on the morning of day 12, three days after the third 16.25 mg/kg overdose administration. Histopathology did not reveal a cause of death.[22]

The 240 charts from the 48 drug-free control male and female mice in Trial 1 and the 72 control male and female mice in Trial 2 were analyzed to examine whether elements in the experimental design, including single-cage housing, were potentially confounding factors. The analyses, which will be reported elsewhere, revealed a daily increase in ocular scores in both control and drug treated mice. The increase suggested stress due to the single housing protocol. A logistic regression model was used to adjust for the variations observed for the drug-free control mice in comparing behavioral differences between the buprenorphine-treated and the drug-free control mice. As described below, significant behavioral differences persisted between the buprenorphine-treated and control mice.

A comparison of the observed behaviors of the drug-treated and control groups revealed that for all days in both trials, there was a general trend that the mice treated with buprenorphine exhibited decreased motor activity. This was recorded by the observer as lower exploratory behavior. The analyses of the motor scores from Trial 1 suggested that drug-treated mice were less active on all days; however, the difference was not significant. This same pattern was supported by data from Trial 2, which involved more mice and greater statistical power. Combining the data from both trials resulted in a statistically significant difference ($p = 0.0001$) between the buprenorphine-treated mice and the drug-free controls (Table 3). Although gender differences were noted in the analyses of the control mice, the data in Table 4 demonstrate that the decreased motor activity observed in the drug-treated group as compared to the control group persisted in the subgroup analyses and was significant in male ($p = 0.0005$) and in female mice ($p = 0.0012$).

In comparison to the motor score differences between control and buprenorphine-treated mice, ocular signs were a more sensitive indicator of behavioral differences in males and females. Data for the combined trials, shown in Table 5, demonstrate a significant difference in the drug-treated mice behavior versus controls, $p = 0.0001$. The significant trial differences noted in the analyses of the control mice persisted in the subgroup analyses of the data. As shown in Table 6, the drug-treated mice in Trial 1 had significantly greater ocular scores compared to the control mice, ($p = 0.0003$). Trial 2 shows a similar difference ($p = 0.0001$) between eyes closed behavior in the drug-treated mice versus controls.

**Table 1: Dose, Harvest, and Observation Schedules for TAS Trials 1 and 2**

| | Trial Day | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Single Dose: Trial 1 | | | | | | | | |
| **No. Mice** | **Day: 1** | **2** | **3** | **4** | **5** | | | | |
| **per Dose** | 32 | | | | | | | | |
| **per Harvest** | | 16* | | 16** | | | | | |
| **Charts AM** | | 32 | 16 | 16 | | | | | |
| **Charts PM** | 32 | 16 | 16 | 16 | | | | | |
| | Repeat Dose: Trial 2 | | | | | | | | |
| **No. Mice** | Day: 1 | 2 to 3 | 4 | 5 | 6 | 7 | 8 | 9 to 11 | 12 |
| **per Dose** | 32 | | 32 | | | | 16 | | |
| **per Harvest** | | | | | 16* | | | | 16** |
| **Charts AM** | | 32 | 32 | 32 | 32 | 16 | 16 | 16 | |
| **Charts PM** | 32 | 32 | 32 | 32 | 16 | 16 | 16 | 16 | |

***weight, hematology, clinical chemistry**
****weight, hematology, clinical chemistry, coagulation panel, organ weight, histopathology**

**Table 2: Parameters evaluated daily by clinical observation**

| | Morning (AM) | Afternoon (PM) |
|---|---|---|
| **General** | **X** | **X** |
| **Respiration** | **X** | |
| **Motor Activity** | **X** | **X** |
| **Tremors** | **X** | |
| **Ocular Findings** | **X** | **X** |
| **Nasal Findings** | **X** | |
| **Behavioral Signs of Distress** | | **X** |
| **Coat Appearance** | **X** | **X** |

**Table 3: Drug Effect on Motor Scores, Combined Trials**

| Motor Score | Control | Drug* |
|---|---|---|
| **NOA: No. (%)** | **148 (61.7)** | **105 (43.8)** |
| **Rapid, Darting: No. (%)** | **49 (20.4)** | **45 (18.8)** |
| **Hunched, Lethargic: No. (%)** | **6 (2.5)** | **5 (2.1)** |
| **Hunched, Motionless: No. (%)** | **37 (15.4)** | **85 (35.4)** |

***p = 0.0001**

**Table 4: Drug Effect on Motor Scores by Gender**

| Motor Score: Male | Control | Drug* |
|---|---|---|
| **NOA: No. (%)** | **85 (70.8)** | **54 (45.0)** |
| **Rapid, Darting: No. (%)** | **13 (10.8)** | **23 (19.2)** |
| **Hunched, Lethargic: No. (%)** | **2 (1.7)** | **1 (0.8)** |
| **Hunched, Motionless: No. (%)** | **20 (16.7)** | **42 (35.0)** |
| **Motor Score: Female** | **Control** | **Drug**** |
| **NOA: No. (%)** | **63 (52.5)** | **51 (42.5)** |
| **Rapid, Darting: No. (%)** | **36 (30.0)** | **22 (18.3)** |
| **Hunched, Lethargic: No. (%)** | **4 (3.3)** | **4 (3.3)** |
| **Hunched, Motionless: No. (%)** | **17 (14.2)** | **17 (35.9)** |

*p = 0.0005; **p = 0.0012

**Table 5: Drug Effect on Ocular Scores, Combined Trials**

| Ocular Score: (%) | Control | Drug* |
|---|---|---|
| **NOA: No. (%)** | **186 (77.5)** | **128 (53.3)** |
| **Eyes closed: No. (%)** | **53 (22.1)** | **112 (46.7)** |
| **Crusty Secretions: No. (%)** | **1 (0.4)** | **0 (0.0)** |

*p = 0.0001

**Table 6: Drug Effect on Ocular Scores by Trial**

| Ocular Score: Trial 1 | Control | Drug* |
|---|---|---|
| NOA: No. (%) | 82 (85.4) | 59 (61.5) |
| Eyes closed: No. (%) | 3 (13.6) | 37 (38.5) |
| Crusty Secretions: No. (%) | 1 (1.0) | 0 (0.0) |
| **Ocular Score: Trial 2** | **Control** | **Drug**** |
| NOA: No. (%) | 186 (77.5) | 128 (53.3) |
| Eyes closed: No. (%) | 53 (22.1) | 112 (46.7) |

*p=0.0003; **p=0.0001

## 4. DISCUSSION

Information about the effects of postsurgical analgesia on the behavior of surgically-treated mice and rats is limited. Studies designed to investigate this behavior would be challenged by the need to inject mice and rats at 6-8 hour intervals for 2-3 days for effective pain management. This approach to pain management could add additional stress to an animal recovering from an already invasive operative procedure.[8] The use of food and water-based analgesia may provide an alternative, less distressing analgesia protocol.[31-33] A long-acting subcutaneous injection of an opiate also promises another mechanism to monitor behavior without introducing the stress of multiple injections.

This analysis of data obtained from trials of a buprenorphine-based drug for mice offers information regarding opiate analgesia and observations of stress. The present report describes a retrospective analysis of cage-side observational data collected from a regulatory study for the approval of a new veterinary pharmaceutical drug, an extended-release buprenorphine analgesic for subcutaneous injection. Histopathology and clinical laboratory results from preclinical studies of the drug, both in powdered form and liquid suspension, have been published and demonstrate the safety and efficacy of the lipid-bound buprenorphine.[22,28] The TAS trials for both drugs included protocol-driven behavioral observations by an experienced observer blind to the treatment groups. The forms were designed for the entry of numerical grading of the extent to which pain or stress was present across nine parameters, including mouse respiration, nasal and skin appearance, fur appearance, motor activity, ocular activity (closed or open eyes), behavior suggesting stress (i.e. aggressiveness), presence of tremors, and surgical site erythema, edema, or infection. Ratings were made on scales ranging from 1-3 to 1-6, depending on the parameter. Although the threshold sensitivity for nociceptive interference with normal behavior remains unknown,[35] certain signs of pain and distress are generally agreed upon. Healthy mice and rats have clean, sleek, well-groomed fur, good skin and eye color, and retain somewhat stretched body positions. They are alert, socially active, inquisitive, tend to explore the cage perimeter, and make quick, smooth movements.[35-38] Nonetheless, because of their small size, it can be difficult to detect signs of pain and distress in rodents.[39] They may conceal outward signs of pain as a survival mechanism.[40] Since first proposed by Morton and Griffiths the use of a numerical scoring system across five independent variables (a. changes in body weight; b. external appearance; c. measurable clinical signs; d. unprovoked behavior; and e. behavioral responses to external stimuli) has become the widely accepted method of objectively assessing pain and distress in laboratory animals.[41] As further described by The University of Newcastle Animal Care and Ethics Committee, a higher numerical score in each observational category on Morton and Griffith's scale represents a more significant sign of pain or stress.[42]

Reviews of the observational data from the TAS trials of the drug in its powder form were uninformative regarding the question of behavioral changes. Signs of sedation were not observed in surgically-treated BALB/c mice given 3, 9, and 15 mg/kg of long acting lipid-encapsulated buprenorphine powder.[28] On average, male and female mice treated with a five-fold overdose of the drug powder (15 mg/kg) appeared to be more lethargic than mice treated with the no-drug control powder. However, cross trial comparisons can be limited. Observers in the trials with the powdered form of the drug were male. A female observer recorded behaviors in the present study. Sorge et al. reported that exposure of mice and rats to male but not female experimenters produces pain inhibition.[43]

In the present trial, with an injectable form of the drug at a slightly higher dose of 16.25 mg/kg, differences between buprenorphine-treated mice and controls were observed in two of the nine parameters examined: ocular activity and motor activity. Statistical analysis revealed that mice who received buprenorphine displayed more closed eyes behavior and were less active than the mice who received control vehicle injections.

It has been proposed that closed eyes and decreased movement may be present in animals experiencing pain or stress.[44-45] Yet, the two groups examined in this report had no other physiological, respiratory, or behavioral differences indicative of pain or stress.[22] Physical appearance and number of abnormal paw movements were comparable between groups, which further suggest that the observed decreases in motor and ocular activity were not clear indicators of distress. We believe that the differences in these two parameters more likely reflected sedation, an expected side effect of moderate buprenorphine overdosing.

The daily ocular findings were not consistently significant across the trials, which we believe supports the conclusion that rather than exhibiting pain or stress, mice that received buprenorphine experienced the drug's side effect of increased lethargy. Within the system of motor activity, the general trend was that drug-treated mice exhibited lower exploratory behavior. In each trial separately, mice displayed more eyes closed behavior on Day 4 (data not shown). The lack of consistently significant findings across all days, despite pharmacokinetic evidence that mice were experiencing clinically significant analgesia,[22] further

supports the conclusion that observed findings did not reflect mice in pain or stress.

When taken together with previous studies of an implantable long-acting buprenorphine powder,[28] the results of the current trials suggest that adverse behavioral effects of buprenorphine analgesia are limited, and seen more frequently at high doses delivered as an injectable suspension rather than an implanted powder. Nonetheless, several factors limit these conclusions. Signs of pain and distress in rodents may be more evident by comparing the appearance and behavior of the traumatized animal with its non-traumatized cage mates to better detect possible abnormalities. For example, a mouse in pain may not move as quickly as cage mates or may isolate, demonstrating reduction in activity, exploratory behavior, and interaction.[46]

A number of studies have examined the beneficial effects of environmental enrichment and social stimuli (i.e. cage mates) on rodent recovery from invasive experimental procedures such as surgery, as well as disease and neurological disorders.[47-50] Social housing of mice may ameliorate acute physiological stress responses,[51] and also influence rodents' perception of pain. Compared to male mice with cage mates, singly housed male mice had increased defecation and visceral sensitivity following electrode implantation.[52] Female mice housed together exhibited less self-administration of analgesics after cecal manipulation and laparotomy procedures.[53] Additionally, social housing of mice might allow for better observations to be made, as the behavior and physical appearance of treated mice could be closely compared to that of non-treated cage mates. This could help determine whether there were clearly discernable differences that would suggest pain and distress. The beneficial effects of enriched environments and social housing on pain, distress, and healing consequently have been widely proposed.[11, 15, 30,54,55] As seen in Table 5, over the study's duration, mice within the drug-free control groups from both trials exhibited increasing signs of stress (Day 1 vs Day 5). The single cage protocol and lack of environmental enrichment thus may have been a confounding factor. However, this did not change the drug's effects on the animals (Tables 3-6). Future research should consider incorporating environmental enrichment or using multiple mice per cage to examine whether this has an impact on behavioral and physical signs.

Results from the current study provide evidence that modest, but significant, signs of opiate therapy can be apparent in mice treated with a high dose of long-acting buprenorphine analgesia. It seems reasonable to consider whether such signs could be detected at clinical doses using more sensitive markers.[17,37] and with new tools to evaluate analgesia.[56, 57] Alternative methods of laboratory animal observation, such as automated video recording parameters, which have been shown to have comparable accuracy to human observation, also may detect behavioral differences at lower dose levels.[58] Future studies might incorporate both observation methods to better determine the frequency and consistency of differences in motor and ocular activity.[58] It is possible that buprenorphine-treated mice exhibited closed eyes and low exploratory behavior only during the specific observation times. Using video recording observation might also allow for better analysis of behavioral differences such as closed eyes versus squinting over the course of a day.

## 5. CONCLUSION

The parameters evaluated by clinical observation as described in this report support the body of literature demonstrating that buprenorphine can be safely applied in rodent research models that warrant analgesia.[59-61]. The absence of differences across the examined parameters, and the inconsistency of significant differences for motor activity and ocular findings, suggest that a high dose of buprenorphine does not induce behavioral or physical signs that clearly indicate pain or stress. Rather, taking into consideration the pharmacological properties of buprenorphine as a partial agonist at the mu-opioid receptor, the observed decrease in exploratory behavior and tendency to have closed eyes during observation times are considered to indicate drowsiness, a mild and expected side effect of buprenorphine dosing. When opioid analgesics are an experimental variable, one must consider analgesic side effects when interpreting the behavior of laboratory mice.

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