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The Effects of Subcutaneous Ketamine on Postlaparotomy Analgesia and Behavior in Female Sprague–Dawley Rats (*Rattus norvegicus*)

Rachael E Alionhart, DVM, MS,* McKayla M Carlson, DVM, Alina R White, ALAT, Kim E Saunders, DVM, DACLAM, and Jennifer H Kopanke, DVM, PhD, DACLAM

Multimodal analgesia provides superior pain control compared with single-agent analgesic approaches. However, certain analgesic drug classes such as NSAIDs and opioids may be contraindicated in some studies due to their mechanisms of action, highlighting the need for alternative analgesic options. Little information is available as to the efficacy of alternative supplementary analgesics in laboratory rodents. Here, we investigate the impact of ketamine as an adjunctive analgesic postlaparotomy in 32 female Sprague–Dawley (SD) rats. Rats received either 4 mg/kg Meloxicam in Extended-Release Polymer (Melox-ER) or 1 mg/kg Buprenorphine Base in Extended-Release Polymer (Bup-ER), along with either ketamine (30 mg/kg SC) or volume-matched saline (n = 8 per treatment group). Postoperative pain behaviors were assessed via video scoring at 30, 90, and 150 min postoperatively, and cage-side evaluations were performed in-person at 3, 6, 12, 24, 32, 48, 56, and 72 h postoperatively. Rat grooming behavior, assessed by the grooming of fluorescent oil from the nape, was evaluated as an indirect method of assessing analgesic efficacy. All rats that received ketamine exhibited higher activity levels, reduced incisional licking, and fewer pain-associated behaviors than nonketamine-treated rats during the initial 90-min postoperative period. Rats that received Melox-ER demonstrated fewer pain-associated behaviors than Bup-ER-treated rats in the acute postsurgical period, regardless of ketamine treatment. Rats treated with Bup-ER took significantly longer to groom fluorescent oil from their fur compared with Melox-ER-treated rats. Our study demonstrates that ketamine confers significant analgesic effects for at least 90 min postoperatively and supports the use of fluorescent oil grooming transfer scores as a method for evaluating postoperative analgesia.

Abbreviations and Acronyms: Bup-ER, Buprenorphine Base in Extended-Release Polymer; bup-ket, Bup-ER with ketamine treatment group; bup-sal, Bup-ER with saline treatment group; CBS, composite behavior score; Melox-ER, Meloxicam in Extended-Release Polymer; melox-ket, Melox-ER with ketamine treatment group; melox-sal, Melox-ER with saline treatment group; NSAID, non-steroidal anti-inflammatory drug; RGS, rat grimace scale; SD, Sprague–Dawley

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Introduction

The minimization of pain and distress through the provision of appropriate perioperative analgesia is critical for animal welfare. Pain can be acute or chronic, somatic or visceral, and nociceptive, inflammatory, or neuropathic in nature.[1–7] Separate classes of analgesics work via different mechanisms of action to modulate signals at various locations throughout the pain pathway. Drug combinations that use more than one class of analgesic to target multiple parts of the pain pathway result in multimodal analgesia. Multimodal analgesia increases the efficacy of perioperative pain control through synergistic mechanisms while minimizing negative side effects resulting from high doses of unimodal drug administration.[1,3,4,8–11]

Two first-line analgesics that are commonly used in combination for postoperative pain management in laboratory animals are buprenorphine, an opioid, and meloxicam, an NSAID. Buprenorphine is a partial μ-agonist opioid and has antagonistic effects at δ and κ receptors.[2] It targets both somatic and visceral pain and may modulate some inflammatory pathways.[2] While buprenorphine has been shown to be an effective postoperative analgesic in rat laparotomy models,[12–16] it also may play a role in opioid-induced postoperative hyperalgesia[17,18] and has been associated with negative side effects such as pica.[19–26] Buprenorphine primarily acts to inhibit the perception of pain signals but also impacts pain modulation and transduction.[11] Meloxicam is an enolic acid-derived NSAID with preferential cyclooxygenase-2 inhibition. Meloxicam acts both centrally and peripherally through modulation and transduction of the pain signal[11] and provides better somatic pain control than visceral.[2] Opioid-NSAID combinations are commonly used to provide multimodal analgesia in rodent surgical models. However, for certain models, one analgesic drug class or another may be contraindicated for clinical or study-specific reasons. In these cases, the administration of an adjunctive analgesic from a different drug class may be necessary to provide sufficient pain management and maintain animal welfare.

Ketamine, a noncompetitive NMDA-receptor antagonist, prevents the stimulatory effects of excitatory glutamate in the central nervous system and is a commonly used dissociative anesthetic. It also has analgesic activity. Ketamine modulates pain signals at the level of the spinal cord and decreases central

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Department of Comparative Medicine, Oregon Health & Science University, Portland, Oregon
**Corresponding author. Email: alionhar@ohsu.edu*

sensitization and wind-up of pain. It has been shown to minimize hyperalgesia, allodynia, and spontaneous and neuropathic pain.[11,27–31] Ketamine provides greater relief of visceral pain than somatic.[32] While there are conflicting reports in the human literature regarding ketamine's ability to prevent persistent postoperative pain,[29,33–37] it has been administered subcutaneously at subanesthetic doses for control of depressive behaviors and chronic pain in humans.[38–44] In veterinary medicine, ketamine is commonly administered intramuscularly for sedation or intravenously for induction or intraoperative adjunctive anesthesia and analgesia.[31,45] Studies have also demonstrated that ketamine may play an important role in postoperative pain management by improving outcomes in both dogs[46,47] and sheep.[48] In rodents, ketamine is most often used in combination with an α-2 agonist such as xylazine or dexmedetomidine for noninhalant anesthetic protocols.[31] Ketamine retains its pain-modulating effects at subanesthetic doses[31]; in rats, its antinociceptive action has been observed at doses greater than 25 mg/kg.[28,49–51] Although the half-life of ketamine is less than 24 h,[51,52] administration of parenteral ketamine at doses less than or equal to 25 mg/kg relieves depressive-like behaviors in rats for multiple days.[51,53–55] While ketamine's interactions at opioid and monoaminergic receptors[28,31,37,43,49,56] and its immunomodulatory and antiinflammatory effects[52,55,57–61] are well described, its potential role in pain management plans for rodents and other laboratory animal models remains poorly characterized.

Methods for establishing analgesic efficacy vary across species. Facial grimace scoring and behavioral pain ethograms can be used to evaluate whether animals are painful based on their physical appearance, posture, and activity levels.[62–64] Indirect methods of evaluating behavior include body weight trends and their correspondence to food and water intake, time to incorporate nesting material,[65,66] nest structure,[67,68] and nesting behavior. More recently, a method was developed to evaluate mouse grooming behavior as an indicator of analgesic efficacy.[66] A fluorescent oil was applied to the nape of the neck of mice; mice with lower pain scores groomed the oil more quickly from their skin and fur; however, data are lacking as to whether this grooming transfer assessment can similarly be applied in rats to evaluate their pain levels.

Here, we aimed to determine the clinical efficacy of subcutaneous ketamine at a subanesthetic dose (30 mg/kg) in combination with extended-release (ER) formulations of either buprenorphine or meloxicam as part of a multimodal postoperative analgesic plan in female Sprague–Dawley (SD) rats following experimental laparotomy. We elected to use experimental laparotomy as the painful stimulus, as it is a well-established model for the study of analgesic efficacy and allows for the evaluation of both somatic and visceral pain relief.[14,15,17,23,62,69–71] We hypothesized that analgesic protocols that included ketamine would provide superior postoperative analgesia compared with single-analgesic protocols. A secondary aim was to assess whether latency to fully remove a fluorescent signal from the fur could be used as an indicator of postoperative pain levels in rats.

Materials and Methods

Experimental animals. Thrity-two 8-wk-old female Sprague–Dawley rats [CRL:CD(SD)] were obtained from Charles River Laboratories. Rats were allowed to acclimate at our facility for at least 48 h before any experimental manipulation. Surgeries were performed on the fifth day after arrival. Before experimental manipulation, rats were handled for at least 5 to 10 min each day using a gentling technique to facilitate acclimation to handling. Rooms were maintained at 70 ± 2 °F with 30% to 70% relative humidity. The rooms were maintained on a 12:12-h light:dark cycle.

Rats were free from the following agents: Kilham rat virus, Toolans H-1 virus, rat minute virus, rat parvovirus, pneumonia virus of mice, rat theilovirus, reovirus, Sendai virus, sialodacryoadenitis virus, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, *Pneumocystis* spp, *Aspiculuris* spp, *Syphacia* spp, *Mycoptes* spp, *Myobia* spp, and *Radfordia* spp.

Upon arrival, rats were single-housed in static polycarbonate caging (R20HT; Ancare) with wire cage tops and static microisolation lids. Rats were housed on 1/4-in. pelleted cellulose bedding (BioFresh Performance Bedding; BioFresh) with ad libitum access to food (5L0D PicoLab Laboratory Rodent Diet; LabDiet) and purified, reverse osmosis, chlorinated, autoclaved water in bottles. All rats received a red- or amber-colored rat retreat (Bio-Serve), nylon bone gnawing enrichment (Bio-Serv), and 2 packets of nesting paper (Enviropak; W.F. Fisher and Son).

To facilitate the measurement of food consumption, approximately 30 g of food was provided daily in a small ramekin on the cage floor.

Ethical review. All experimental procedures were approved by the Oregon Health & Science University IACUC and performed in a facility accredited by AAALAC, International. This study adhered to the principles in the *Guide for the Care and Use of Laboratory Animals* and all institutional and federal regulations.

Study design. Before surgery, each rat was assigned to one of 4 experimental treatment groups ($n = 8$ per group): Meloxicam in Extended-Release Polymer (Melox-ER) with saline (melox-sal), Melox-ER with ketamine (melox-ket), Buprenorphine Base in Extended-Release Polymer (Bup-ER) with saline (bup-sal), or Bup-ER with ketamine (bup-ket). Treatment group assignment and the order in which treatment groups underwent the initial midline laparotomy procedure were determined via a random-number generator by a nonblinded individual. No saline-only negative control group was used, as the rat laparotomy model is a well-established and validated pain model.[14,15,17,23,62,69–72]

Following induction of anesthesia, a nonblinded assistant administered an ER analgesic subcutaneously to each rat, either 1 mg/kg Bup-ER ($n = 16$; 1 mg/mL; Wedgewood Pharmacy) or 4 mg/kg Melox-ER ($n = 16$; 2 mg/mL; Wedgewood Pharmacy). Rats subsequently received subcutaneous injections of either 30 mg/kg ketamine ($n = 16$; 100 mg/mL stock of ketamine HCl [Dechra Veterinary Products]; diluted to 5 mg/mL in 0.9% sodium chloride USP [Pfizer]) or volume-matched 0.9% sodium chloride ($n = 16$; Hospira; MWI Animal Health) before being removed from anesthesia. Additional ketamine (10 mg/kg) or volume-matched saline was administered subcutaneously at 24 and 48 h postoperatively.

An individual who was blinded to all treatment groups performed all postoperative observations and injections. Injections were performed in awake rats by gently holding them against the body of the handler and administering the entire volume subcutaneously into the right or left flank.

Midline laparotomy. A midline laparotomy was performed to elicit the postsurgical pain model. All surgeries were performed by a single surgeon blinded to treatment group. Surgeries were performed between 0700 and 1200. Rats were anesthetized via isoflurane (Fluriso; VetOne; MWI Veterinary Supply) delivered in 100% oxygen. Rats were induced at 4% isoflurane and maintained on a nosecone at 1.25% to 2.5% isoflurane throughout surgery.

Before surgery, a 1.5 × 1.0-in. patch of fur was clipped between the caudal neck and dorsal scapulae for injection of either Bup-ER or Melox-ER. Ketamine or volume-matched saline was administered subcutaneously at the left or right flank. The surgical site was clipped and aseptically prepared with 4.0% chlorhexidine gluconate (Hibiclens; Molnlycke Health Care) and 70% isopropyl alcohol. Following transfer to the operating table, rats were draped (Glad Press'n Seal).

A 2.0- to 3.0-cm midline abdominal incision was made. A sterile cotton-tipped applicator or the surgeon's sterilely gloved finger was moved throughout the abdomen for 120 s. The abdominal muscle layer was closed with 4-0 monofilament polydioxanone suture (One-Dox; VetOne) in a simple continuous pattern. The skin layer was closed with sterile 9-mm wound clips (Braintree Scientific) or a continuous intradermal suture pattern with 4-0 monofilament polydioxanone. Tissue glue (cyanoacrylate surgical adhesive; VetOne) was used to cover knots that were unable to be fully buried under the skin.

Before removal from isoflurane anesthesia, 2 mL of warmed saline was administered subcutaneously. All rats were recovered with heat support and were returned to their home cage once they were sternal and ambulatory.

Behavioral observations and pain assessment. ***Video scoring.*** To monitor the acute postoperative period without disturbing the animals, cages were placed in view of a camera (2.7K Video Camera; Shenzhen Seree Technology) within the housing room immediately after anesthetic recovery. Each camera was mounted on a tripod approximately 3 ft from the front of the cage at a distance similar to that of a human observer. Cages were oriented with the broad side facing the camera, allowing each camera to record 2 rats simultaneously. Pain behaviors were subsequently scored by a blinded observer at 30, 90, and 150 min following completion of surgery. The blinded observer retroactively watched 10 min of video at each time point and counted the frequency of discrete occurrences of pain behaviors as previously described,[15,69,70] termed the composite behavior score (CBS). If an animal was obscured from the camera, the duration of observation was extended until a total of 10 min of behavior was evaluated.

Evaluated behaviors included the following: (1) back-arch: a cat-like arching of the spine; (2) writhe: contraction of flank muscles; and (3) stagger/fall/wobble: a loss of balance, seen either as fully falling or as quicker than normal movement of the feet. Back-arch and writhe behaviors were combined into a single score due to the propensity for rats to perform behaviors simultaneously while making turns within rat retreats. Duration of time (1) performing cephalic and nonabdominal caudal grooming (licking paws or dorsal flank, grooming face/head); (2) abdominal licking (associated with the incision); (3) eating food or bedding; (4) interacting with nesting material; and (5) general inactivity (not moving or performing any behaviors) was also noted. As some analgesics have been noted to decrease grooming behaviors,[15,71] cephalic grooming was grouped with nonabdominal body licking to differentiate from incisional-directed licking and self-trauma; incisional-directed licking was counted as such even if it progressed from sequential grooming motions.[73]

In addition to evaluation of the CBS at 30, 90, and 150 min, orbital tightening, as described in the rat grimace scale (RGS), was evaluated,[64] along with overall posture ("Posture/Appearance") and behavior ("Behavior/Activity") scores from the pain ethogram as outlined in Figure 1. These ethogram components were specifically selected as they could be readily evaluated via video recording. Other components of the RGS could not be reliably evaluated on video scoring due to the angle and distance from the camera. Components of the pain ethogram that specifically required the handler to interact with the animal ("Weight" and "Response to External Stimuli") were not evaluated during the video scoring component.

Cage-side assessments. Animals were assessed cage-side by the same blinded observer at 3, 6, 12, 24, 48, 56, and 72 h after cessation of anesthesia. For approximately 5 min, each rat was

Score	Weight
0	<5% body weight lost
1	5-10% body weight lost
2	10-15% body weight lost
3	>15% body weight lost

Score	Posture/Appearance
0	Normal: smooth coat, minimal to absent hunching, absence of porphyrin staining
1	Slightly rough coat, mild hunching, minimal porphyrin staining
2	Rough coat, moderate hunch, moderate porphyrin staining, mild dehydration
3	Unkempt, moderate to severe hunch, porphyrin staining, moderate dehydration

Score	Response to External Stimuli
0	Normal behavioral responses for expected conditions (i.e. reaching into cage, handling, stroking back or head); mild response to firm palpation of incision acceptable
1	Minor depression or response exaggeration; mild response on gentle palpation of incision
2	Moderate signs of abnormal responses; hyperactivity, jerky or spastic behavior, vocalization or moderate response to gentle palpation of incision
3	Animal either overreacts to external stimuli or is minimally responsive to non-responsive

Score	Behavior/Activity
0	Normal activity, behavior, breathing, and ambulation; unbothered by incision
1	Slightly abnormal behavior or slightly quiet; slight increase in respiratory rate or effort, occasional licking at, or porphyrin at, incision, slightly abnormal gait
2	Quiet to lethargic, increased respiratory rate or effort, decreased activity, back-arching or writhing, consistent licking or porphyrin at incision, guarding abdomen, obviously altered gait
3	Lethargic, severely increased respiratory rate and effort, minimal activity

Figure 1. Pain ethogram. Ethogram criteria were used in conjunction with the rat grimace scale (RGS)[64] to assess postoperative pain. A blinded observer scored each animal for each ethogram category at 3, 6, 12, 24, 32, 48, 56, and 72 h after recovery from anesthesia. The total score was calculated as the sum across all categories. Only the "Posture/Appearance" and "Behavior/Activity" components from the pain ethogram were evaluated during the retroactive video scoring assessment, as these could be readily observed via video and did not require the handler to directly interact with the animals.

evaluated for facial grimace score using RGS,[64] pain ethogram score (Figure 1), and other behaviors to assess postoperative pain. Briefly, the RGS evaluates 4 characteristics of a rat's facial appearance (orbital tightening, nose/cheek flattening, ear changes, and whisker change) on a scale of 0 to 2, with a higher score corresponding to a greater pain intensity.[64] Using our pain ethogram, the total score was calculated as the sum across all categories. Animals receiving a total pain ethogram score of greater than 5 or an averaged RGS of greater than 1 warranted secondary assessment by veterinary staff. Animals with a pain ethogram subscore of 3 in any single category warranted secondary assessment by veterinary staff and rescue analgesia.

To discourage rats from sleeping through the full 5-min evaluation, all animals were roused by brief handling at the beginning of the evaluation period. CBS was only assessed in the acute postoperative period via video scoring, as the presence of a human observer during cage-side assessments was considered to be a confounding variable in the expression of the CBS behaviors. Body weight, food consumption, and nest complexity were also recorded through at least 72 h postsurgery. All fecal pellets were collected through 5 d postsurgery to assess for the occurrence of pica.

Fluorescent oil application and grooming transfer. To establish each animal's baseline grooming transfer behavior, an initial grooming transfer test was performed on rats 48 h after arrival at the facility before any surgical manipulation. For baseline application, rats were induced for the midline laparotomy procedure and maintained on a nosecone for a total of 15 min before oil application and recovery. A second grooming transfer assessment was then performed during the immediate postoperative period to determine whether there were changes to the animal's grooming behavior after surgery. For both the baseline and postoperative applications, 100 µL of nontoxic fluorescent powder suspended in mineral oil (Glo Germ Oil; Glo Germ) was applied via pipet to the skin between the ears of each rat as it recovered from isoflurane anesthesia.

Fluorescent signal was subsequently scored at 3, 6, 12, 24, 32, 48, 56, and 72 h postapplication using a 5-point scale (Figure 2). Signal strength was detected with a handheld UV light (UVL 1006; Glo Germ).

Food collection and weight. Immediately postoperatively, a fresh ramekin of approximately 30 g of feed pellets was provided in each cage, with the exact weight recorded for each rat. To measure food consumption at each subsequent postoperative cage-side observation, all intact food pellets were manually collected from the cage and weighed. Every 24 h, all uneaten feed was discarded and replaced with 30 g of fresh food pellets (exact weight recorded in each case); this was repeated through 7 d postsurgery. Baseline food consumption was determined for each rat by calculating the average of the amount of food consumed each day for 3 d before surgery.

Nest complexity scoring. Nesting was scored at each cage side assessment using an adapted scoring algorithm[68] as an indirect method of evaluating postoperative pain management. We modified our scoring system to accommodate for nest-building material being provided contained within cotton bags, as well as the presence of rat retreats. Here, a score of 0 reflected untouched nesting material or material being piled, a score of 1 reflected minimal manipulation or a disorganized arrangement such as material strewn across the cage, a score of 2 indicated a disorganized nest with loose or flattened materials and minimal central indentation or the presence of nesting material within the rat retreat, and a score of 3 reflected a well-organized circular nest structure with an obvious central depression or greater than two-thirds of nesting material being present within the shelter. Following each cage-side assessment, all nesting material was gathered and moved into the corner of the cage by the observer, effectively resetting all scores to zero unless manipulation by the rat occurred. This "reset" of the nesting materials was performed at each assessment (3, 6, 12, 24, 48, 56, and 72 h postoperatively).

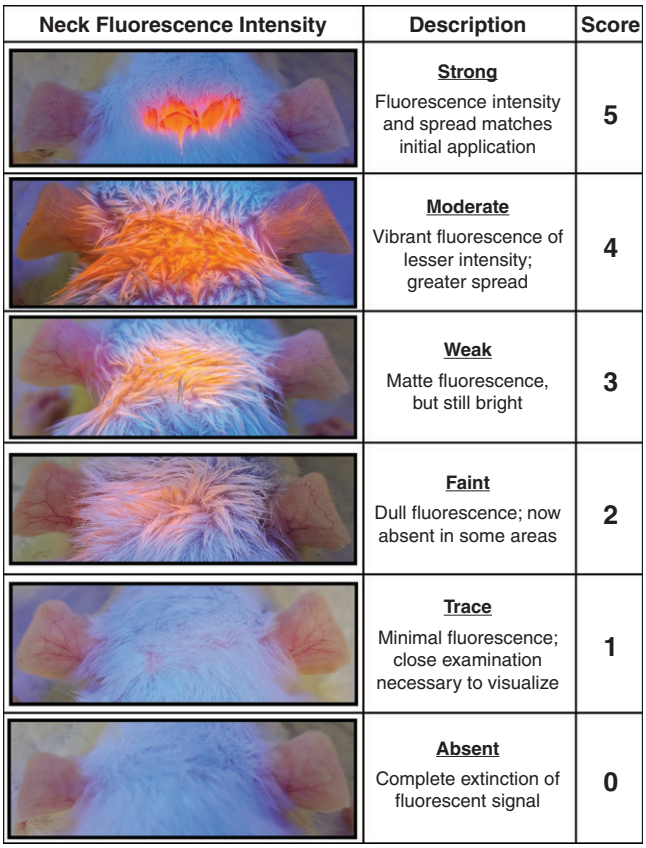

Figure 2. Grooming transfer test scale. Intensity and spread of fluorescence were assessed under UV light for signal extinction from the dorsal neck over a 72-h period. Contrast of all images was enhanced by 10% to correct for light-balancing of photography.

Statistical methods. GraphPad Prism (Version 10.4.1; GraphPad) was used for all statistical analyses. Data are expressed as means ± SD. A P value of 0.05 or less was considered statistically significant.

A mixed-effects model with a Geisser-Greenhouse correction was used to accommodate missing values. The within-subject effect was defined as time, and the between-subjects effect was defined as the analgesic treatment group (melox-sal, melox-ket, bup-sal, bup-ket, and baseline where applicable). Post hoc pairwise comparisons were performed using the Tukey adjustment for multiple comparisons. Q-Q plots were evaluated to confirm normal distribution of data. Group size was modeled after similar studies and in consultation with the Oregon Health & Science University Biostatistics and Design Program and was considered appropriate based on the "resource equation" method.[15,74]

Results

Three rats from the bup-ket group demonstrated significant self-mutilation with accompanying incisional dehiscence at 12 h (n = 1) and 24 h (n = 2) postoperative. Of these, 1 rat underwent incisional repair and received rescue analgesia, while 2 were euthanized. One rat each from the bup-sal and melox-ket groups experienced dehiscence at 24 and 96 h, respectively, and

was euthanized. Of all rats with dehiscence, the rats euthanized at 12 and 96 h had their incisions closed with wound clips ($n = 2$), while all rats that were euthanized at 24 h had skin closed via intradermal suture pattern ($n = 3$); there was no correlation between closure method and dehiscence. Data collected from these rats up until their time of withdrawal were included in data sets. All euthanized rats were necropsied and showed no gross abnormalities of abdominal organs attributable to surgical procedures, although histologic confirmation was not performed. Of note, variable amounts of bedding were observed in the gastrointestinal tract of all individuals.

One rat from the bup-ket treatment group had a protracted anesthetic recovery and thus was excluded from all 30-minute time points.

Behavioral observations and pain assessment. ***Video scoring.*** The frequency of back arching and writhing was not significantly different at any time point for the melox-sal treatment group (Figure 3A). Rats in both the melox-ket and bup-sal treatment groups had higher frequencies of back arching and writhing at 30 min than at 150 min ($P < 0.05$). Bup-ket-treated rats had higher frequencies of back arching and writhing at 90 min compared with animals in both the bup-ket and bup-sal groups at 150 and 90 min, respectively ($P < 0.01$).

Frequency of staggering, falling, and wobbling were significantly higher for groups that received ketamine compared with saline (Figure 3B) at both 30 min ($P < 0.0001$ for Melox-ER; $P < 0.01$ for Bup-ER) and 90 min ($P < 0.05$ for Melox-ER; $P < 0.01$ for Bup-ER). There were also significant differences between every time point within both ketamine treatment groups ($P < 0.01$ for both melox-ket and bup-ket), with the overall frequency of staggering decreasing over time for ketamine-administered rats.

Orbital tightening (Figure 3C) was significantly increased for saline-treated groups compared with ketamine-treated groups at 30 min ($P < 0.01$ for Melox-ER; $P < 0.001$ for Bup-ER) and 90 min ($P < 0.05$). In addition, the degree of orbital tightening was significantly greater at 30 min compared with 150 min for rats treated with melox-sal ($P < 0.01$).

Posture/Appearance scores were higher for saline-treated rats compared with ketamine-treated rats at 30 min (Figure 3D). Specifically, Posture/Appearance scores were significantly higher for rats in the melox-sal treatment group at 30 min than at 90 and 150 min ($P < 0.01$), as well as when compared with melox-ket-treated rats at 30 min ($P < 0.0001$). Posture/Appearance scores for rats in the bup-sal treatment group were significantly higher than those in the bup-ket group at both 30 and 150 min postsurgery ($P < 0.05$).

Behavior/Activity scores were higher for saline-treated rats at multiple time points compared with ketamine-treated rats (Figure 3E). Specifically, scores were significantly higher for rats in the melox-sal treatment group at 30 min than at 150 min ($P < 0.05$), as well as compared with the melox-ket treatment group at 30 min ($P < 0.05$). Behavior/Activity scores were significantly higher for rats in the melox-ket treatment groups at 30 min compared with 90 min ($P < 0.01$) and 150 min ($P < 0.05$). Behavior/Activity scores for rats in the bup-sal group were significantly higher at all time points compared with rats in the bup-ket treatment group ($P < 0.05$ at 30 and 90 min, $P < 0.01$ at 150 min). In addition, Behavior/Activity scores were significantly higher for rats within the bup-ket group at 30 min compared with 150 min ($P < 0.05$).

The duration of time that rats were inactive was higher for rats in both saline treatment groups at 30 min compared with ketamine treatment groups (Figure 3F). Specifically, melox-sal-treated rats were significantly less active at 30 min than at 150 min ($P < 0.05$), as well as compared with melox-ket-treated rats at 30 min ($P < 0.0001$). Inactivity levels were higher for bup-sal-treated rats at 30 min compared with bup-ket-treated rats ($P < 0.0001$). Bup-ket-treated rats had significantly higher inactivity levels at 150 min than at 30 min ($P < 0.05$) or 90 min ($P < 0.05$).

Nonincisional grooming, including that of the paws, head, and dorsal flank, was not significantly different for any treatment group at any time point (Figure 3G). Incisional licking was most likely to occur in rats of the bup-sal treatment group (Figure 3H). Specifically, at 150 min postsurgery, bup-sal-treated rats spent a significantly longer time licking the area around the incision than at 30 min ($P < 0.05$), and when compared with bup-ket-treated rats at 150 min ($P < 0.05$). In addition, at 30 min postsurgery, saline-treated rats spent significantly more time licking around abdominal incisions than ketamine-treated rats ($P < 0.05$).

Cage-side assessment. RGS scores did not differ between treatment groups at any time point (Figure 4A). Across the 72-h postoperative period, RGS scores did not differ within treatment groups for melox-sal, melox-ket, and bup-ket groups. Within the bup-sal treatment group, however, rats had lower RGS scores at 56 and 72 h compared with 6 and 48 h ($P < 0.05$), with a significantly higher score at 48 h compared with 24 h ($P < 0.05$).

Pain ethogram score did not differ between treatment groups at any time point (Figure 4B), but within each treatment group, rats generally had higher scores closer to surgery compared with lower scores further from surgery. Within the melox-sal group, rats had significantly lower pain ethogram scores at 72 h than at 3, 6, 12, and 32 h, and at 48 h than at 32 h ($P < 0.05$). For melox-ket-treated rats, animals had significantly lower pain ethogram scores at 72 h than at 56 h ($P < 0.01$). Within the bup-sal group, rats had significantly lower pain ethogram scores at 48 and 72 h than at 12 h ($P < 0.05$). Finally, for bup-ket-treated rats, animals had significantly lower scores at 72 h than at 6, 12, and 32 h, and at 56 h than at 12 h ($P < 0.05$).

All rats lost weight within 6 h following surgery compared with baseline, but on average, rats that received Bup-ER as part of their analgesic plan had higher weights than those that were treated with Melox-ER through 32 h postsurgery (Figure 5). Specifically, rats treated with bup-sal had higher weights than those treated with melox-sal at 6 h ($P < 0.05$), 12 h ($P < 0.05$), 24 h ($P < 0.01$), and 32 h ($P < 0.05$), while rats treated with bup-ket had higher weights than those treated with melox-ket at only 24 and 32 h ($P < 0.05$). Bup-ER-treated rats weighed significantly less than presurgical baseline weights at only 2 points each: bup-ket at 3 h ($P < 0.05$) and bup-sal at 6 h ($P < 0.05$). Meanwhile, melox-ket treated rats weighed significantly less compared with presurgical baseline at all time points through 32 h ($P < 0.01$), with melox-sal-treated rats weighing significantly less at 3, 6, and 32 h postsurgery ($P < 0.05$ at 3 h and 32 h; $P < 0.01$ at 6 h). However, the amount of food eaten did not differ between treatment groups at any time point.

Neck fluorescence score. Neck fluorescence scores were higher for Bup-ER-treated rats than for Melox-ER-treated rats compared with their presurgical baseline (Figure 6). Rats in the melox-sal treatment group did not show significant delays in grooming compared with baseline at any time point, while rats in the melox-ket treatment group only demonstrated a significantly higher fluorescence intensity score at 3 h postsurgery compared with baseline ($P < 0.01$). In contrast, bup-ket-treated rats maintained higher fluorescence intensity at 3 h ($P < 0.0001$),

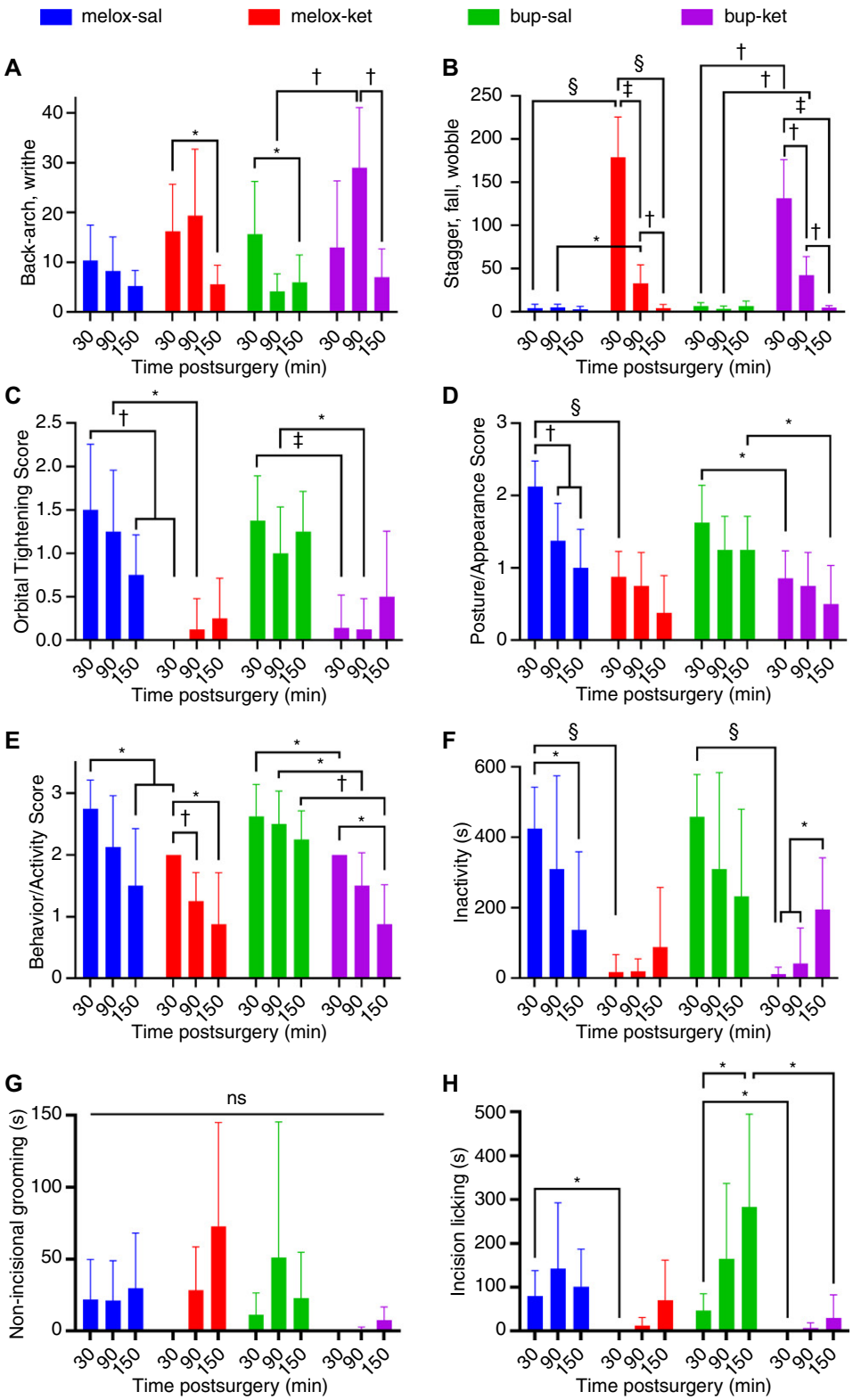

Figure 3. Behavioral assessments of postoperative pain evaluated during retroactive video scoring for each treatment group (melox-sal, melox-ket, bup-sal, and bup-ket) in female Sprague–Dawley rats at 30, 90, and 150 min postsurgery. Shown are the cumulative frequency of (A) back-arching and writhing; (B) staggering, falling, and wobbling; scores for (C) orbital tightening; scores for (D) Posture/Appearance and (E) Behavior/Activity; and duration in seconds of (F) inactivity, (G) nonincisional grooming, and (H) licking at incision. Values are shown as mean ± SD. Significant differences between values: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$; ns, no significance.

6 h ($P < 0.01$), 12 h ($P < 0.001$), and 24 h ($P < 0.05$) postsurgery, and bup-sal-treated rats maintained higher fluorescence intensity through 32 h postsurgery ($P < 0.001$ at 3 h, $P < 0.01$ at all other time points).

Rats administered Bup-ER took longer to reduce their fluorescence scores compared with rats administered Melox-ER. Rats in the bup-ket group had higher fluorescence scores at 3 h ($P < 0.05$) and 12 h ($P < 0.01$) postsurgery compared with

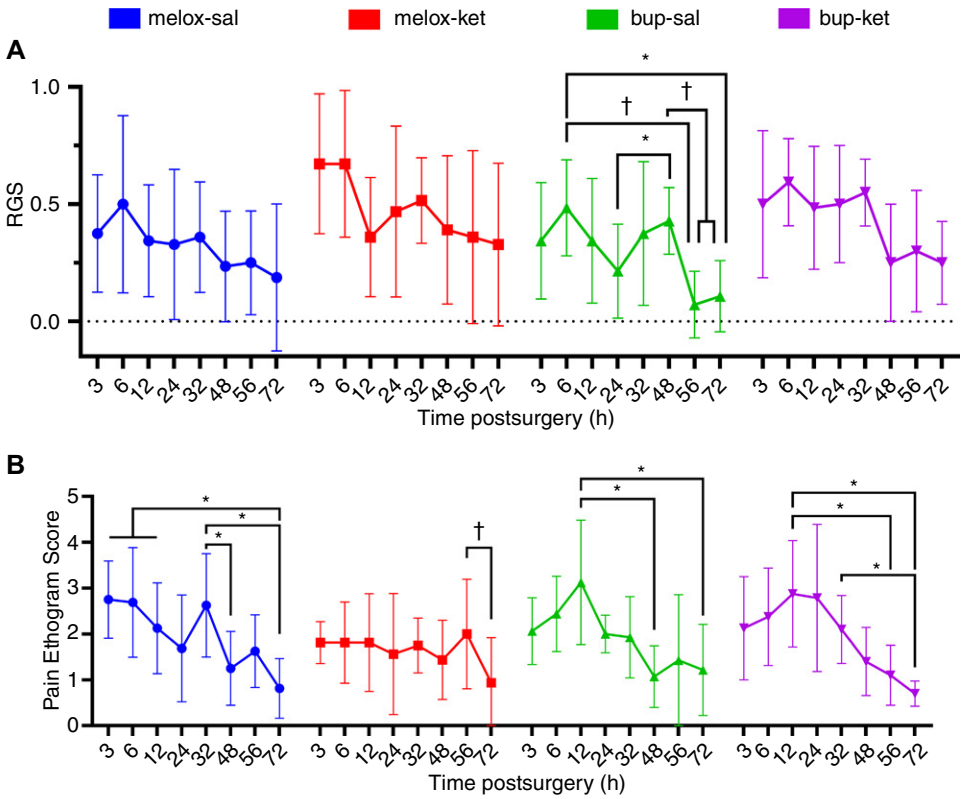

Figure 4. (A) RGS and (B) pain ethogram scores for each treatment group (melox-sal, melox-ket, bup-sal, and bup-ket) evaluated cage side at 3, 6, 12, 24, 32, 48, 56, and 72 h postsurgery. Values are shown as mean ± SD. Significant differences: *, $P \leq 0.05$; †, $P \leq 0.01$.

the melox-ket group. Rats in the bup-sal group retained higher fluorescence intensity scores at 3 h ($P < 0.05$), 6 h ($P < 0.01$), 12 h ($P < 0.01$), 24 h ($P < 0.05$), and 32 h ($P < 0.05$) compared with the melox-sal group.

Nest building and complexity score. Rats in the melox-ket group had the quickest return to baseline nest score at 6 h postsurgery compared with the other groups, which returned to baseline levels by 12 h postsurgery (Figure 7). In addition, rats receiving Melox-ER created significantly more complex nests than rats receiving Bup-ER at 6 h postoperatively ($P < 0.05$ for saline treated; $P < 0.01$ for ketamine treated).

Discussion

Here, we investigated the use of ketamine as an adjunctive analgesic to manage postlaparotomy pain in female SD rats. Based on ketamine's pain-modulating properties, we hypothesized that it could be an efficacious component of a multimodal pain management plan, particularly in circumstances where key

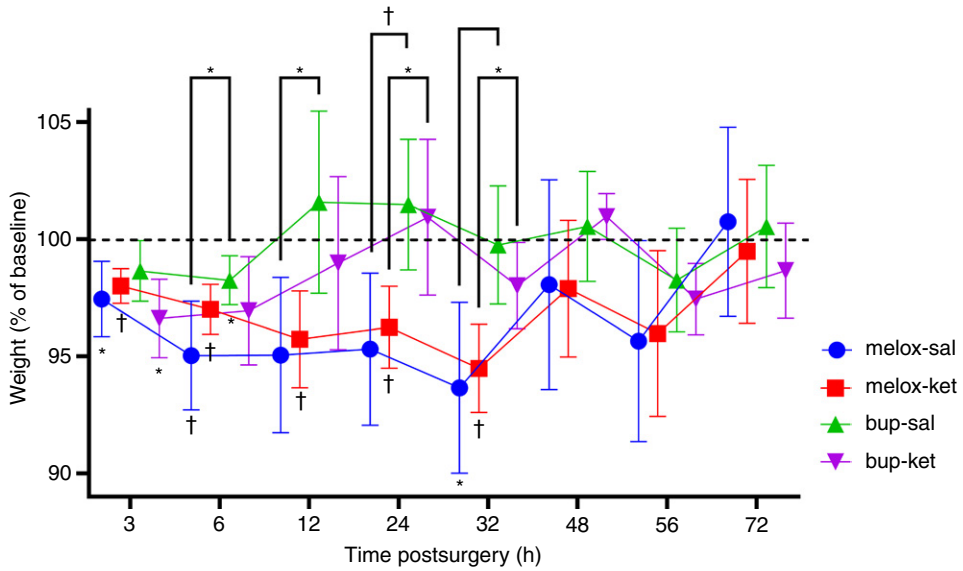

Figure 5. Weight measured as a percentage of baseline for each treatment group (melox-sal, melox-ket, bup-sal, and bup-ket), through 72 h postsurgery. Baseline weight was measured on the morning of surgery, represented by the horizontal dashed line at 100% of baseline; symbols beneath data points represent significant differences from baseline. Values are shown as mean ± SD. Significant differences: *, $P \leq 0.05$; †, $P \leq 0.01$.

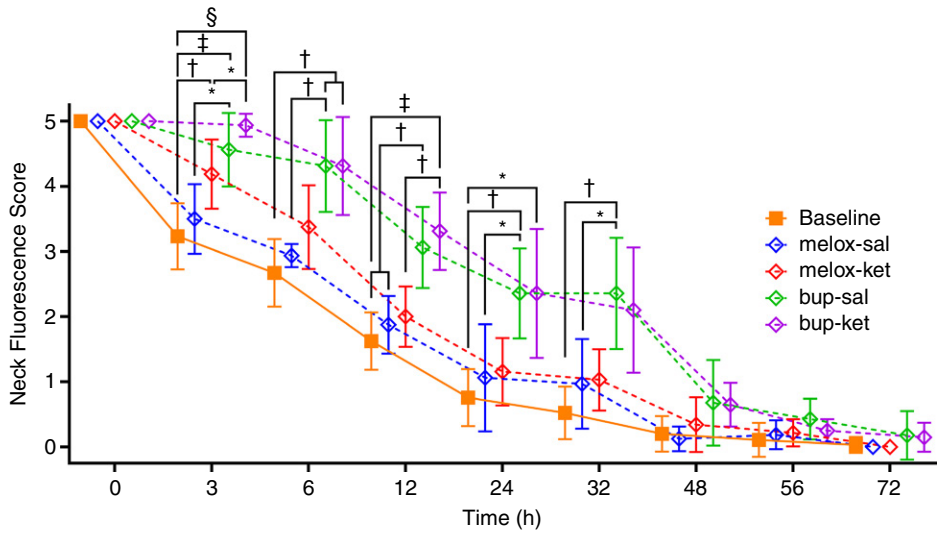

Figure 6. Neck fluorescence intensity score over 72 h postsurgery for each treatment group (melox-sal, melox-ket, bup-sal, and bup-ket) and compared with baseline. Baseline scores were collected 72 h before surgery and fully removed by rats 24 h before surgery. For both baseline and postoperative applications, fluorescent oil was applied to the nape of the neck while animals were under isoflurane anesthesia. Values are shown as mean ± SD. Significant differences between values: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$.

classes of systemic analgesics (NSAIDs, opioids) may be contraindicated for clinical or experimental reasons. Administration of Melox-ER or Bup-ER was supplemented by either subcutaneous ketamine or volume-matched saline, and postoperative pain was assessed via video scoring and cage-side assessments that included evaluation of RGS, CBS, and physiologic parameters. We opted to not incorporate a no-analgesia group due to the well established nature of the laparotomy pain model and the overall goal of our study, which was to evaluate the response of animals receiving ketamine as part of their postoperative pain management compared with those receiving unimodal analgesia. Our findings demonstrate that ketamine confers significant analgesic effects for at least 90 min postoperatively. In addition, cumulative data indicate that Melox-ER-treated rats demonstrate fewer pain-associated behaviors than Bup-ER-treated rats during the acute postsurgical period, regardless of ketamine administration.

Overall, our study found that female SD rats that received ketamine (melox-ket and bup-ket groups) appeared less painful during the immediate recovery period than those that did not receive ketamine. Animals receiving ketamine had significantly lower scores corresponding with pain responses than the saline controls at several time points during the first several hours following surgery. Ketamine-treated animals demonstrated increased locomotion and staggering behaviors, likely attributable to the dissociative effects of this drug. While it is possible that these behaviors may have masked more subtle pain signals, we believe ketamine had a true analgesic effect. Specifically, buprenorphine-treated rats had equal frequencies of back-arching/writhing regardless of ketamine administration, but animals that did not receive ketamine were significantly less active than those that did. In addition, saline-only groups had significantly higher rates of incisional licking compared with ketamine-treated animals. The significantly higher Posture/Appearance scores, Behavior/Activity scores, and orbital tightening scores for animals receiving saline instead of ketamine are difficult to write off as a side effect of ketamine's dissociative effects. The combination of Melox-ER and ketamine may confer additional benefits in the immediate postoperative period compared with Bup-ER with ketamine, as evidenced by higher activity scores in rats treated with melox-ket compared with those that received bup-ket. As ketamine modulates the pain signaling pathway differently than both opioids and NSAIDs, this emphasizes the importance of multimodal analgesia for animal welfare when controlling postoperative pain.[11]

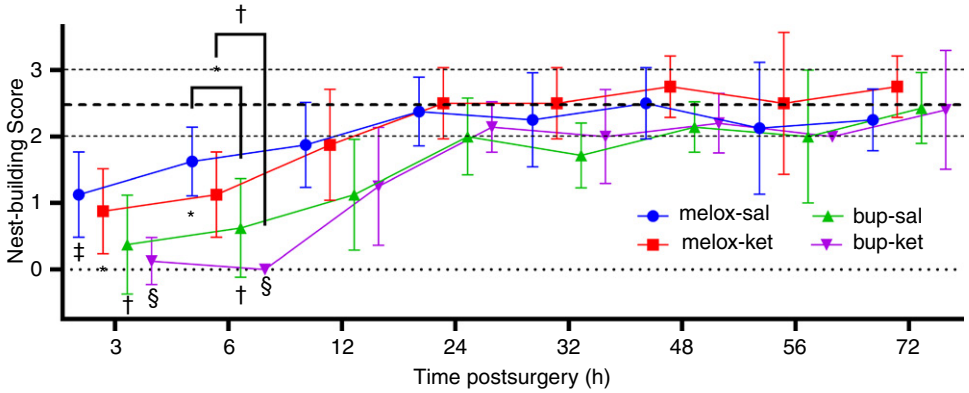

Figure 7. Mean nest-building score for each treatment group (melox-sal, melox-ket, bup-sal, and bup-ket) evaluated cage-side at 3, 6, 12, 24, 32, 48, 56, and 72 h postsurgery. Values are shown as mean ± SD; the thick and thin horizontal dashed lines represent the mean and SD of baseline nest-building scores, respectively. Symbols beneath data points represent significant differences from baseline. Significant differences between values: *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$.

Pain ethogram and RGS scores were not significantly different between treatment groups at any time point, although animals tended to have higher ethogram scores closer to surgery, with scores decreasing over time. While it has been shown that isoflurane anesthesia alone can increase RGS scores,[75] one potential explanation for these findings is that each of the drug combinations provided roughly equivalent levels of analgesia, although more subtle differences between groups may have been detectable with larger group sizes. Another contributing factor may have been the familiarity of rats with the primary observer, which could have influenced cage-side behavior due to anxiolytic effects. Tickling, a handling technique that mimics rat play, has been shown to decrease anxiety in stressed rats surrounding procedures.[76] While no tickling was performed during this study, animals were well acclimated to handling, and many rats acted quite tame and affiliative during cage-side assessments. Moreover, interactions during the cage-side assessments may have been particularly rewarding to the rats as all were single housed for the duration of the experiment. Physiologic measures (weight), indirect methods of behavioral evaluation (food consumed, nest complexity, and grooming transfer), and video scoring bypassed the potential impacts of human influence on observed behaviors.

Rats consumed the same amount of food regardless of treatment group. However, rats that received buprenorphine, either in combination with ketamine or alone, weighed more than meloxicam-treated animals for approximately the first 32 h after surgery. While this could be a true sequelae of surgery, it is possible that an increased consumption of bedding induced by buprenorphine administration may be a contributing factor,[19–26] with pica behavior linked to nausea in rats.[77] Evidence of pica was frequently observed in rats in our study regardless of treatment group, although it was subjectively most prominent in the animals that received buprenorphine as part of their analgesic plan. Additional factors related to buprenorphine administration, such as decreased gastrointestinal motility and urinary retention, also may have contributed to higher weights in buprenorphine-treated animals, although other alternatives such as higher fluid retention or consumption cannot be ruled out. Our observations suggest that pica may be a common behavior in SD rats that may be exacerbated with buprenorphine and/or ketamine administration.

Nest complexity is not widely used in the assessment of analgesic efficacy in rats, but we observed that most rats in our study interacted with paper manipulanda to create nests. Nest-building quality scores were adapted from a previously described scale,[68] which we modified to account for the presence of a square shelter in the cage. While it is not possible to determine whether differences in nest building and complexity resulted from the presence of discomfort compared with side effects of certain drugs, using our modified scale we found that rats treated with melox-ket returned to baseline nest complexity more quickly than any other group. In addition, rats that received Melox-ER with or without ketamine had higher nesting complexity scores earlier in the postoperative period than those treated with Bup-ER. Other factors that may have contributed to the robust nesting behavior observed in animals in our study overall may include single housing, ample floor space (153 in.^{2}), and the provision of 2 packets of nesting material.

An indirect method of evaluating animal welfare and pain mitigation using the rats' natural grooming behavior was also investigated. A fluorescent oil applied to the nape of anesthetized rats was assessed under UV light for signal extinction over 72 h. When reapplied postlaparotomy, Bup-ER-treated rats took significantly longer to reduce the fluorescent intensity and return to baseline signal extinction compared with Melox-ER-treated rats, regardless of ketamine treatment. We found that melox-ket-treated rats had a slight delay in their return to baseline grooming levels compared with the melox-sal group, likely due to a lack of grooming during the immediate postoperative period where animals were constantly ambulating around the cage as a side-effect of ketamine. Our findings confirm that rat grooming behavior is impacted in the postoperative period and that analgesic selection may have a variable effect on grooming. Studies[71] have shown that buprenorphine administered in the absence of a surgical procedure can result in decreased grooming. Whether the grooming differences observed between the Melox-ER- and Bup-ER-treated rats in the postoperative period are attributable to the characteristics of the analgesics themselves or animal pain levels warrants further study; regardless, this is a compelling early finding that rat grooming transfer may be a helpful tool to indirectly measure analgesic efficacy in future studies.

We observed several instances of self-trauma at the incisional site, resulting in dehiscence and removal from the study. Animals treated with Bup-ER were overrepresented, with this tendency to self-traumatize exacerbated by the addition of ketamine in several cases. Numerous studies have described pica,[19–26] staple-removal,[17] and self-trauma[19,20,24] in rats administered buprenorphine or ER buprenorphine. There also appears to be an interaction between buprenorphine and the background strain, with 1 study[26] observing pica in SD but not Long-Evan rats, while another study[24] documented pica and self-trauma in SD but not Lewis rats. Our findings corroborate these studies, with pica and self-trauma occurring in some of our buprenorphine-treated SD rats.

None of the rats treated with bup-ket that demonstrated incisional dehiscence reacted painfully on superficial or deep palpation of incisions, with 1 of these rats severely self-mutilating her abdominal skin beyond the extent of the original incision. An opioid-ketamine interaction may have reduced the protective sensations of nociceptive pain.[6,78] As ketamine enhances opioid analgesia, lower doses of both ketamine and Bup-ER when used in combination may still provide adequate analgesia while resulting in fewer negative side effects.

The doses of ketamine and frequency of administration used in this study were based on studies characterizing the antidepressant effects of ketamine in rats. The initial 30-mg/kg perioperative dose was selected for the well-described antinociceptive effects of ketamine at doses greater than 25 mg/kg,[28,49–51] and the 2 subsequent doses of ketamine at 10 mg/kg were adapted from protocols studying its antidepressive effects in rats.[51,53–55] As ketamine's role as a postoperative analgesic is otherwise poorly characterized in rats, these studies offered a valid starting point for better understanding the role of this agent in rat analgesic protocols. The additional doses we provided at 24 and 48 h postoperatively did not appear to have an appreciable impact on the measures evaluated in this study. Future studies are warranted to investigate optimal ketamine dosages and dosing frequencies to minimize negative side effects and to determine whether redosing is necessary.

Bup-ER and Melox-ER both appear to have sufficient activity as single-analgesic protocols, but we saw greater benefits to the use of Melox-ER for the SD rats in our study: Melox-ER-treated rats demonstrated no difference in grooming transfer compared with baseline, had improved nest complexity scores compared with baseline and Bup-ER-treated rats, and did not exhibit increased incisional licking at later time points.

The meloxicam-treated animals demonstrated fewer signs of discomfort in the immediate postoperative period (decreased orbital tightening, better Posture/Appearance scores, better Activity/Behavior scores, and lower duration of inactivity). Melox-ER-treated rats that also received ketamine as part of their postoperative pain management appeared the most comfortable out of all treatment groups through 72 h postsurgery.

While ketamine was found to have positive pain management qualities for postoperative animals in our study, certain drawbacks should be considered at the doses we employed. First, we found that animals that received ketamine demonstrated unusually high levels of activity in the acute postoperative period, with increased frequencies of staggering, falling, and wobbling during the 90-min postoperative period. While such signs can be attributable to pain, we believe these behaviors are instead related to the dissociative effects of ketamine. Therefore, ketamine may be contraindicated for certain orthopedic and neurologic models due to the side effect of hyperactivity, as well as for any behavioral paradigms that should be performed soon after anesthetic recovery; however, a subanalgesic but antidepressive dose of ketamine at 10 mg/kg could be considered. Due to the high incidence of self-trauma observed in our buprenorphine-treated rats, we recommend caution and rigorous monitoring if using Bup-ER as the primary analgesic for laparotomy models in young female SD rats, particularly if injectable anesthetic and/or analgesic combinations include ketamine. Future directions of study should include optimization of ketamine dosages and the interval of ketamine administration for postoperative pain management in SD rats, as well as evaluation of its performance in standard algesiometry assays. Finally, this study was only performed using female SD rats. Future studies should investigate ketamine's analgesic effects in male rats, as well as in other strains and genetic backgrounds.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Generalizability/Translation

The findings of this study are likely to generalize to other species, as the mechanisms of actions that ketamine uses to modulate pain are conserved between species. While primarily being used pre- and intraoperatively in veterinary medicine, ketamine has also already shown promise in human studies for controlling chronic pain postoperatively.

Protocol Registration

This protocol was not externally preregistered before the initiation of the study.

Data Availability

Available upon request.

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