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Evaluation of Pain Assessment Techniques and Analgesia Efficacy in a Female Guinea Pig (*Cavia porcellus*) Model of Surgical Pain

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Guinea pigs (*Cavia porcellus*) are a frequently used species in research, often involving potentially painful procedures. Therefore, evidence-based recommendations regarding analgesia are critically needed to optimize their wellbeing. Our laboratory examined the efficacy of carprofen and extended-release (ER) buprenorphine, alone and as a multimodal combination, for relieving postsurgical pain in guinea pigs. Animals were assessed by using evoked (mechanical hypersensitivity), nonevoked (video ethogram, cageside ethogram, time-to-consumption test), and clinical (weight loss) measurements for 96 h during baseline, anesthesia–analgesia, and hysterectomy conditions. In addition, ER buprenorphine was evaluated pharmacologically. Guinea pigs treated with a single analgesic showed increased mechanical sensitivity for at least 96 h and indices of pain according to the video ethogram for as long as 8 h, compared with levels recorded during anesthesia–analgesia. In contrast, animals given both analgesics demonstrated increased mechanical sensitivity and behavioral evidence of pain for only 2 h after surgery compared with anesthesia–analgesia. The cageside ethogram and time-to-consumption tests failed to identify differences between conditions or treatment groups, highlighting the difficulty of identifying pain in guinea pigs without remote observation. Guinea pigs treated with multimodal analgesia or ER buprenorphine lost at least 10% of their baseline weights, whereas weight loss in carprofen animals was significantly lower (3%). Plasma levels for ER buprenorphine exceeded 0.9 ng/mL from 8 to 96 h after injection. Of the 3 analgesia regimens evaluated, multimodal analgesia provided the most effective pain control in guinea pigs. However the weight loss in the ER buprenorphine–treated animals may need to be considered during analgesia selection.

Abbreviation: ER, extended-release

Pain is a complex experience comprising sensory and emotional components that affects quality of life. Much of pain research using animal models is performed on rodents,[42] a group of prey species that do not readily display overt signs of pain.[3,41] Guinea pigs are a species that is protected by the Animal Welfare Act.[54] In 2015, more than 50,000 guinea pigs in the United States were used in a teaching or research setting in which they had the potential to experience pain.[55] Opioids and NSAID are commonly used to alleviate pain in this species. Despite the frequent use of these analgesics in studies anticipated to induce more than slight or momentary pain, very few studies[2,15,52] have looked into their efficacy in guinea pigs. Analgesic regimens for guinea pigs appear to be based primarily on anecdotal experience or parallels with other species. Therefore, the creation of evidence-based recommendations for analgesia in guinea pigs is a critical area of refinement needed for this species.

The primary goal of the current study was to evaluate the efficacy of common postoperative analgesics in guinea pigs by using nonevoked, evoked, and clinical measures. More specifically, we chose to evaluate the ability of carprofen and extended-release (ER) buprenorphine, either individually or in a multimodal combination, to alleviate postoperative pain after hysterectomy. Prior studies conducted in rat and rabbit surgical models identified significant benefits of multimodal analgesia over single-agent therapy.[9,21] Therefore, our hypothesis was that guinea pigs undergoing multimodal analgesia would show the least difference between anesthesia–analgesia and surgery conditions, thus minimization of the effects of pain, in all evoked, nonevoked, and clinical assessments of pain.

A secondary goal of this study was to compare the use of nonevoked and evoked measures of pain in postoperative guinea pigs. Pain assessments in rodents have historically focused on testing evoked measures that evaluate reflex responses, such as mechanical hypersensitivity with von Frey filaments or thermal sensitivity with tail-flick and hot-plate tests. More recently, these approaches have been criticized for their oversimplification of the pain experience and, ultimately, poor translatability to the human pain experience.[12,43] Thus, there has been a shift to evaluate nonevoked outcomes of pain in animals to better recapitulate the clinical realities in human medicine.

Nonevoked measures evaluate the effect of pain on the animal's performance of spontaneous behaviors or activities, thus functioning as a surrogate for the influence of 'day-to-day pain' in human patients. The outcome measures of these novel tests are whether and to what degree the animal performs a task or behavior. To date, some of these tests have involved evaluating the frequency or duration of well-described spontaneous behaviors, such as through prescribed ethograms,[14,50,57] facial grimace scoring,[31,53] or the performance of spontaneous activity including exploratory behaviors,[38,39,59] wheel running,[1,11,28] weight bearing,[22] burrowing,[25,26,44] and nesting.[20,25,27,45,48] Because nonevoked measures often are nonspecific to pain, their use requires careful evaluation of baseline behaviors to

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provide a contextual foundation for any subsequent postsurgical change.[41] We hypothesized that nonevoked measures of pain would provide a more clinically relevant representation of the global postoperative pain experience of guinea pigs and enable evaluation of the efficacy of different analgesic treatments.

By exploring these goals, we hoped to improve pain detection and alleviation in guinea pigs and to develop pain assessment tools that more accurately translate pain findings to other species.

Materials and Methods

Animals. Intact female Hartley guinea pigs (n = 24; weight, 450 to 500 g; age, approximately 5 to 7 wk) SPF for Sendai virus, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, and guinea pig adenovirus were acquired from Charles River Laboratories (Saint-Constant, Quebec, Canada, and Kingston, NY) and maintained in an AAALAC-accredited animal facility (University of Michigan, Ann Arbor, MI). The animal housing and experimental protocol was approved in advance by the University of Michigan IACUC. Guinea pigs were pair-housed in plastic guinea pig rack drawer caging (Allentown Caging, Allentown, PA) with paper shaving bedding (R and R Animal Bedding, Lapeer, MI) in a temperature-controlled room (21 ± 2 °C) on a 12:12-h light:dark cycle. Animals were provided with huts (Guinea Pig Hut, Bioserv, Flemington, NJ), autoclaved cardboard tubes, free access to food (5025 Guinea Pig Diet, LabDiet, St Louis, MO) and automated water and received timothy hay once daily in their home cage. Body weights were collected daily on weekdays by using a pediatric scale.

Upon arrival, guinea pigs were acclimated 5 d each week for 2 wk. Acclimation involved weighing, gentle handling and interaction with female lab members (VO, KS, SA) for 10 to 20 min twice daily, spending gradually increasing time to 60 min in behavioral assessment cages, and exposure to all behavioral testing equipment. Behavioral assessment cages are as described in a previous study.[14] Animals were acclimated to gentle touching of their abdomens to decrease nonpain-associated reactiveness to physical contact during von Frey testing. To minimize inconsistencies, the same personnel performed all experimental manipulations and scoring, described in more detail in the pain assessment sections below.

Experimental design and conditions. Each guinea pig was randomized into 1 of 3 analgesic treatment groups: ER buprenorphine, carprofen or multimodal. Given that animals were pair-housed, they may or may not have been housed with another animal of the same treatment. Each animal acted as its own control, undergoing baseline, anesthesia–analgesia, and surgery conditions. A flow chart summarizing each animal's experimental manipulation is provided in Figure 1. The baseline condition did not involve any experimental manipulation beyond performing the various pain assessments described later. The anesthesia–analgesia condition was included to control for the drug effects of the analgesics and anesthetics as well as all the nonpainful components of surgery, including transportation to surgical suite, anesthesia induction, surgical preparation, and recovery. The surgery condition consisted of all of the components just listed plus a hysterectomy procedure. Comparison of these 3 conditions allowed for the isolation of surgical pain-specific differences in behavior and nociception. All animals were assessed by a blinded observer during each condition over serial time points at 1 to 2, 7 to 8, 23 to 24, 31 to 32, 47 to 48, 71 to 72, and 95 to 96 h. For the anesthesia–analgesia and surgery conditions, these time points began after analgesic administration; the analgesic was administered immediately before surgery, which was conducted between 0900 and 1300. This sampling scheme allowed for 2 time points, 7 to 8 and 31 to 32 h, during the dark cycle when guinea pigs are most active. Each experimental time point consisted of cageside assessments and videorecording of spontaneous behavior, followed by von Frey and time-to-consumption testing. Animals were returned to their home cages after experimental sessions, where they had free access to food and water. A washout period of at least 7 d occurred between anesthesia–analgesia and surgery conditions to permit full recovery after the anesthesia–analgesia condition. During the washout period, animals underwent acclimation procedures as described earlier.

Anesthesia–analgesia. Guinea pigs were placed singly in autoclaved polycarbonate cages with corncob bedding for transportation to and from the procedure room. Anesthesia was induced by using an induction box with 5% isoflurane (VetOne Isoflurane, Boise, ID) carried in 100% oxygen and then switched to a nose cone with 1.5% to 3% isoflurane for maintenance. During anesthesia, pulse oximetry, heart rate, respiratory rate, and temperature were monitored. Anesthetic monitoring was continued throughout the procedure (approximately 50 min of anesthesia total). Animals were kept on an external heat source to help maintain appropriate body temperature. Surgical preparation involved lubricating eyes, emptying cheek pouches, shaving the caudal abdomen from the xyphoid to the pubis, aseptically cleaning the abdomen, and surgical draping. Analgesics were administered shortly after anesthetic induction. Animals received one of 3 analgesic treatments: ER buprenorphine (0.48 mg/kg SC; Animalgesic Laboratories, Millersville, MD); carprofen (4 mg/kg SC every 24 h for 3 d; Rimadyl, Zoetis, Kalamazoo, MI); or multimodal treatment consisting of a single dose of ER buprenorphine (0.48 mg/kg SC) plus carprofen (4 mg/kg SC) every 24 h for 3 d. Because ER buprenorphine had not been evaluated previously in guinea pigs, the dose used was based on allometric scaling from the known mouse and rat doses, whereas the carprofen dose was chosen according to common clinical practice.[7] Animals were switched to 100% oxygen for recovery, remained on external heat support until normothermic, and then returned to their cages once fully ambulatory.

Surgery. Animals underwent anesthesia, analgesia, and surgical preparation as described earlier. In addition, sulfamethoxazole–trimethoprim (30 mg/kg PO) was administered 1 to 2 h prior to surgery to minimize the risk of postoperative infection and was continued twice daily for 7 to 10 d postoperatively. To ensure a consistent surgical stimulus, all surgeries were performed by the same board-certified veterinary surgeon (JN). A small (2 to 3 cm) midline abdominal incision was made midway between the umbilicus and pubis, followed by incision of the linea alba. Each uterine horn was located, exteriorized, and ligated caudal to the ovaries. The broad ligament on each side of the uterine body was bluntly dissected, and any vessels associated with the uterine body were ligated. The uterus was ligated cranial to the cervix, followed by transection of the proximal uterine body and removal of the uterus. The abdomen was closed by using a continuous pattern with absorbable suture on the linea alba and subcutaneous tissue. A subcuticular suture pattern and skin glue (Vetbond Tissue Adhesive, 3M, St Paul, MN) were used to close the skin. No anesthetic or surgical complications occurred, and all animals recovered uneventfully, with the exception of 2 guinea pigs that developed small focal corneal ulcers after the anesthesia–analgesia condition. Cageside and video ethogram scores of these 2 animals were omitted from analysis for the time points when they were affected.

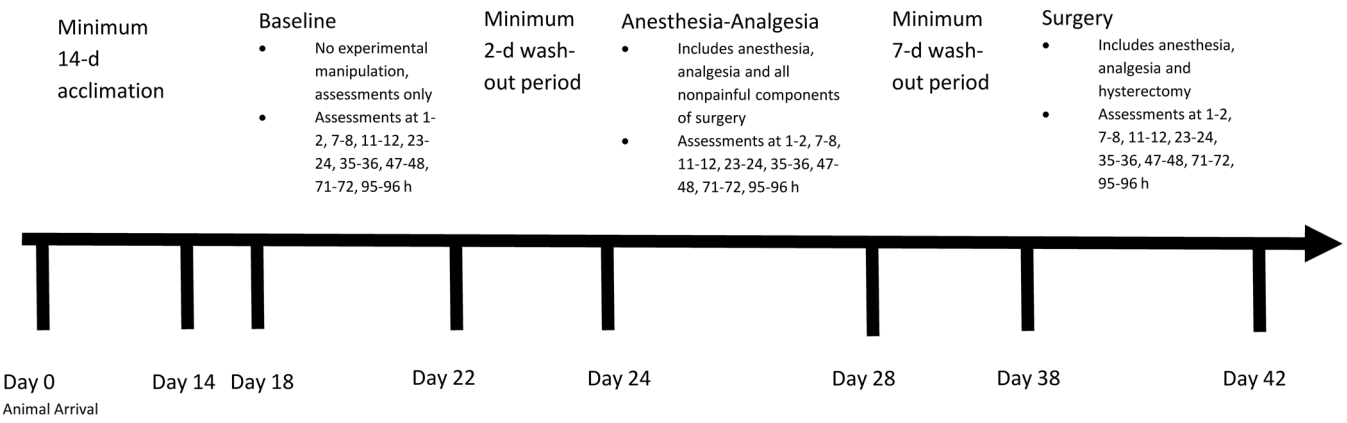

Figure 1. Experimental conditions by each guinea pig.

Both responded to topical treatment and resolved completely prior to surgery.

Nonevoked and evoked pain assessments. ***Video ethogram assessment.*** For each time point, guinea pigs underwent videorecording (HD Everio, JVC, Long Beach, CA) in behavioral assessment cages for 15 min in the absence of a human observer. Videos were randomized and scored by a trained, blinded observer (KS) according to a previously described behavioral ethogram developed in our lab[14] and detailed in Figure 2. Each video was broken into nine 10-s clips (a total of 90 s for each video), and each clip was scored by using a fixed-interval 1–0 sampling method, as previously described.[14] Briefly, each individual behavior was scored as either a 1 when it was performed during the 10-s clip or 0 if it was not performed. These scores were summed across the 9 clips for each behavior to represent a total frequency of observation at each time point. Frequencies of behaviors were analyzed individually and summed within the active or passive categories.

Electronic von Frey measurement. An electronic von Frey probe (IITC Life Science, Woodland Hills, CA) was used to measure mechanical hypersensitivity, which is defined as a decrease in the mechanical nociceptive threshold. The probe was applied perpendicular to the ventral abdomen at 2 locations: the caudoventral abdomen, within a 1-cm radius surrounding the abdominal incision; and the cranioventral abdomen, 1 cm below the xiphoid. The caudoventral abdomen was chosen to reflect postoperative pain as previously described in the evaluation of analgesic efficacy in various veterinary species,[14,17,37] whereas the cranioventral abdomen was selected to control for the animal's response to nonincisional stimulation. Each location was measured 3 times by a blinded observer (VO), with a 5-min washout period between measurements to prevent sensitization. The mean of the 3 measurements was calculated for each location, and then the difference between the mean cranioventral and the mean caudoventral measurements was calculated to determine each animal's nociceptive mechanical threshold (in grams).

Cageside ethogram assessment. Guinea pigs were observed in their home cages by a blinded observer (VO) for 90 s at each time point, prior to video ethogram recording for the presence of the following behaviors: eye closure or orbital fissure (squinting) closure of more than 50%, piloerection of more than 50% of the hair coat, weight shifting, subtle body movement, and coprophagy. Scores (0, absent; 1, present) were recorded for each behavior across the entire cageside observation period and averaged across treatment group and condition.

Time-to-consumption score. After von Frey testing, an acrylic platform (10 × 6 × 2 in.) was placed in the assessment cage with the guinea pig. A cardboard tube stuffed with hay was placed on top of the platform to motivate the guinea pigs to climb on top of the platform. The ends of the tube were folded to increase the time and effort required to access the hay inside, creating a sustained activity which we hoped would recapitulate the sustained effort mice engage in while nesting[27,48] or burrowing.[26] Animals then were given 5 min in the absence of a human observer to climb onto the platform and access the hay. Animals were scored (0, absent; 1, present) for each of the following activities: sitting on the platform, opening the tube, chewing on the tube, and knocking the tube off the platform. Scoring was performed by 2 blinded observers (VO, SA) and summed for each animal at each time point. These scores were then averaged across treatment group and condition.

Pharmacokinetic evaluation. Intact female Hartley guinea pigs (n = 8; weight, 450 to 500 g; age, approximately 5 to 7 wk) with Pinport external access jugular vein catheters and identical SPF status and housing conditions as described earlier were acquired (Charles River Laboratories, Kingston, NY). To assist with recovery from shipping stress, animals were supplemented daily with DietGel CritiCare (Clear H_2O,Westbrook, ME) and were given 3 d to acclimate before they were dosed with ER buprenorphine (0.48 mg/kg SC). The animals were divided into 2 groups to undergo blood collection of 1 mL at baseline, 2, 8, 24, 32, 48, 72, and 96 h postanalgesia time points; blood collection was rotated among animals so that the total amount of blood collected per guinea pig did not exceed 1% of its body weight. Blood was placed in heparinized tubes and centrifuged at 2465 × g for 10 min. Plasma was collected aseptically and the samples stored in –80 °C freezer until shipping. Samples were shipped overnight on ice to the UC Cancer Center Pharmacology Shared Resource Laboratory (Colorado State University, Fort Collins, CO) for evaluation of plasma buprenorphine levels, as previously described and by using liquid chromatography and tandem mass spectrometry of 50 µL plasma.[29,52] Briefly, samples were prepared by using a liquid–liquid extraction method with methyl tert-butyl ether and reconstitution in acetonitrile and reverse-osmosis–purified water. Positive-ion electrospray ionization mass spectra were obtained by using a triple-quadrupole mass spectrometer (AB Sciex Q-Trap 6500, Sciex, Framingham, MA) with a turbo ionspray source interfaced with a Nextera MP Ultra HPLC device with a SIL-30ACMP multiplate autosampler system (Shimadzu, Kyoto, Japan). The lower limit of detection for the analysis was 25 pg/mL. A hypothesized therapeutic

Passive behaviors: frequency increases during pain	
Eyes closed or squinting	Eyelids are closed for a longer duration than a blink or eyes are not open a normal amount (>50%); closure is NOT associated with grooming or coporophagy
Piloerection	Bristling of hairs (that is, fur sticks up) that affects >50% of the body surface caudal to the ears
Weight shifting	Movement of one or more feet to distribute weight without gaining distance
Subtle body movement	Movement of jaw, thorax, or abdomen independent of extremities
Incomplete movement	Animal initiates the act of coprophagy or grooming but abruptly stops
Active behaviors: frequency decreases during pain	
Forward or backward movement	Displacement of at least 2 feet in any direction to gain distance
Body turn	Changing orientation of the head or body more than 180°
Head or neck movement	Head and neck extension or flexion in any direction
Rearing	Vertical elongation of the body, with both forefeet off the ground
Coprophagy	Intentional act of moving head down toward the anus to collect feces

Figure 2. Building on a previously published ethogram for postoperative guinea pigs,[13] we categorized 10 individual behaviors for video behavioral assessment into active and passive categories. The frequencies of the behaviors were analyzed individually as well as summed within a category.

threshold of 1000 pg/mL was selected in light of data from the human literature.[16]

Statistical analysis. Statistical analysis was performed by using Prism 6 for Windows (GraphPad Software, La Jolla, CA). Data were normally distributed and analyzed by using 2-way repeated-measures ANOVA with Tukey multiple comparisons for posthoc testing. This analysis was performed to compare baseline with anesthesia–analgesia conditions and anesthesia–analgesia with surgery conditions across multiple time points. We ran χ^2 tests on the cageside ethogram assessment and time to consumption scores. Results were considered statistically significant when the *P* value was less than 0.05.

Results

Analgesia efficacy. *Buprenorphine*. During the anesthesia-analgesia condition, animals treated with ER buprenorphine demonstrated a 10% or greater body weight loss that was significantly ($P < 0.0001$) lower than the weight loss observed during the baseline condition (Figure 3 A). A similar percentage of weight loss occurred after surgery as well but did not differ from that seen during the anesthesia–analgesia condition. Animals returned to baseline weights or higher between the anesthesia-analgesia and surgery conditions.

For the video ethogram assessment, ER buprenorphine–treated animals displayed a significant ($P < 0.001$) increase in summed passive behaviors after anesthesia-analgesia between 2 and 24 h compared with baseline (Figure 4 A). There was a significant ($P < 0.05$) increase after anesthesia–analgesia compared with baseline in the individual passive behaviors piloerection between 2 and 24 h and subtle body movement between 2 and 32 h as well as at 72 h (Figure 5). Guinea pigs displayed a significant ($P < 0.01$) decrease from baseline in summed active behaviors throughout all time points after anesthesia–analgesia (Figure 4 D). The individual active behaviors of forward or backward movement at 2, 8, and 32 h; body turn at 32 h; head or neck movement at all time points; rearing at 2 and 8 h; and coprophagy at 24, 48 and 72 h were significantly ($P < 0.05$) decreased from baseline after anesthesia–analgesia in ER buprenorphine–treated animals (Figure 5).

After the surgery, ER buprenorphine-treated guinea pigs demonstrated no significant differences in summed active or passive behaviors as compared with the anesthesia–analgesia condition (Figure 4 A and D). However, several individual passive behaviors including subtle body movement at 2 and 96 h and incomplete movement at 32 h were significantly higher ($P < 0.05$) after surgery compared with the anesthesia–analgesia condition (Figure 5). The individual active behavior body turn at 96 h was decreased after surgery in the ER buprenorphine group.

Electronic von Frey evaluation revealed no significant differences between baseline and anesthesia–analgesia conditions. However, after surgery, ER buprenorphine–treated guinea pigs had significantly ($P < 0.001$) increased mechanical hypersensitivity at 32 and 96 h compared with their anesthesia–analgesia condition (Figure 6 A).

Pharmacokinetic evaluation of ER buprenorphine showed plasma levels above 0.9 ng/mL from 8 to 96 h after injection, with peak levels (1.2 ng/mL) at 48 h (Figure 7).

Carprofen. Carprofen-treated guinea pigs displayed a significant ($P < 0.0001$) decrease of approximately 3% body weight after anesthesia–analgesia and surgery conditions but returned to baseline within 48 and 96 h, respectively (Figure 3 B). Between these conditions, surgery showed significantly ($P < 0.0001$) greater weight loss than anesthesia–analgesia from 24 to 72 h.

In the video ethogram assessment, carprofen-treated animals demonstrated only a significant ($P < 0.01$) increase in summed passive behaviors after anesthesia–analgesia compared with baseline at 2 h (Figure 4 B). Specifically, eyes closed or squinting and piloerection at 2 h were significantly ($P < 0.05$) increased after anesthesia–analgesia compared with baseline (Figure 5). Regarding summed active behaviors, animals displayed only a significant ($P < 0.01$) decrease from baseline to anesthesia–analgesia, at 2 h (Figure 4 E). In addition, carprofen-treated animals displayed a significant ($P < 0.05$) decrease after anesthesia–analgesia in the individual active behaviors of forward or backward movement at 24 h and head or neck movement at 2 h (Figure 5). After the surgery condition, animals had significant ($P < 0.05$) increases in summed passive behaviors and significant ($P < 0.05$) decreases in summed active at 8 h compared with the anesthesia–analgesia conditions (Figure 4 B and E). Specifically, the individual active behaviors forward or backward movement at 8 and 32 h, head or neck movement at 8 h, and coprophagy at 2 h were all significantly ($P < 0.05$) decreased after surgery compared with anesthesia–analgesia (Figure 5). Only one individual passive behavior, piloerection, was significantly ($P < 0.05$) increased after surgery at 8 h compared with anesthesia–analgesia.

Evaluation with electronic von Frey testing revealed no differences between baseline and anesthesia–analgesia conditions. However, guinea pigs had significantly ($P < 0.005$) increased mechanical hypersensitivity at 8, 24, 48, and 96 h after surgery compared with the anesthesia–analgesia condition (Figure 6 B).

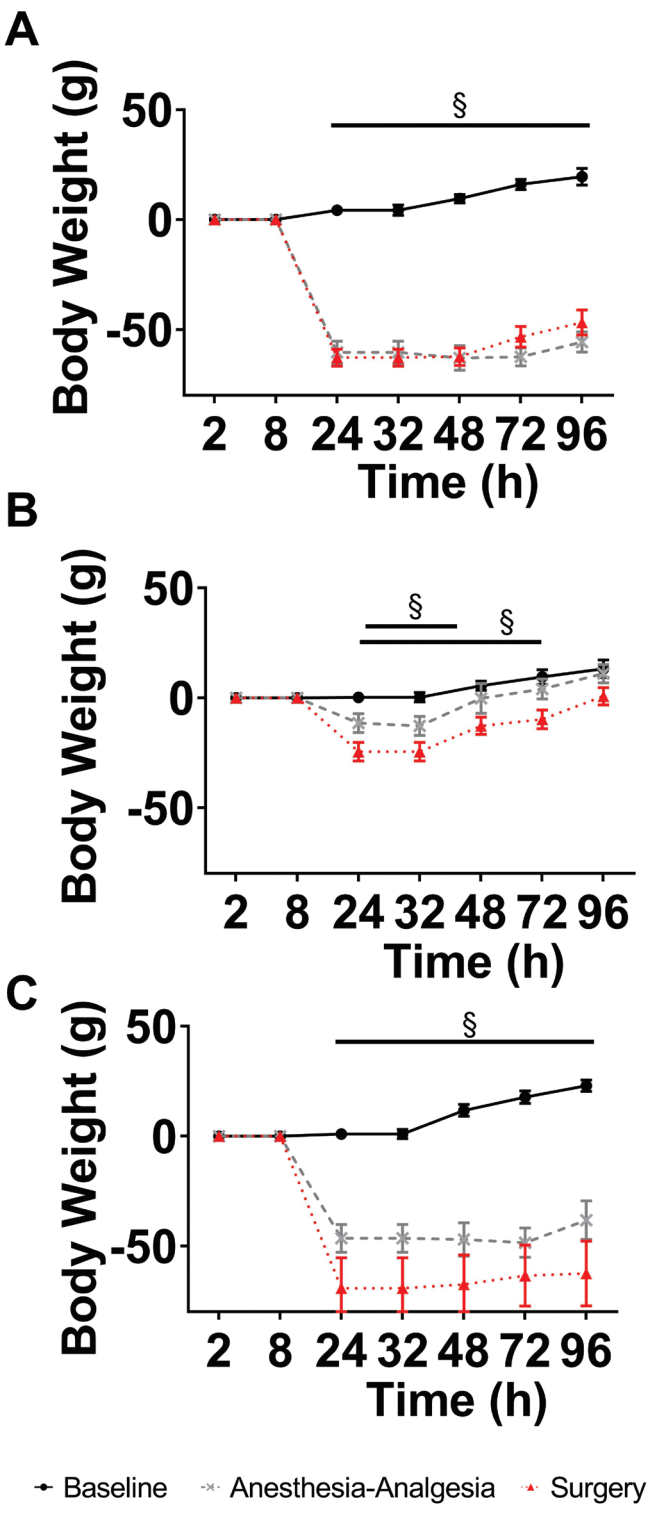

Figure 3. Body weights (mean ± SEM) in (A) ER buprenorphine, (B) carprofen, and (C) multimodal treatment groups during baseline, anesthesia–analgesia, and surgery conditions. §, Value differs ($P < 0.0001$) between anesthesia–analgesia and surgery conditions, thus indicating pain; §, value differs ($P < 0.0001$) between baseline and anesthesia–analgesia conditions, indicating drug-associated effects.

Multimodal analgesia. Similar to ER buprenorphine guinea pigs, the multimodal treatment group displayed a significant ($P < 0.0001$) loss of body weight of greater than 10% during the anesthesia–analgesia and surgery conditions, with body weights during neither condition returning to baseline within the 96-h study period (Figure 3 C). The multimodal group also showed no differences in weight loss between the anesthesia–analgesia and surgery conditions.

Video ethogram assessment revealed significantly ($P < 0.001$) increased summed passive behaviors between baseline and anesthesia–analgesia conditions at 2 and 8 h (Figure 4 C). Individual passive behaviors eyes closed or squinting at 96 h, piloerection at 2 and 8 h, subtle body movement at 8 to 32 h and incomplete movement at 48 h were significantly ($P < 0.05$) increased compared with baseline after anesthesia–analgesia conditions (Figure 5), and there was a significant ($P < 0.01$) decrease in summed active behaviors throughout all time points after anesthesia–analgesia (Figure 4 F). Also similar to ER buprenorphine animals, the multimodal group during the anesthesia–analgesia condition displayed a significant ($P < 0.05$) decrease relative to baseline for the individual active behaviors forward or backward movement at 8, 48, and 96 h, head or neck movement at all-time points, rearing at 8 h, and coprophagy from 8 to 72 h (Figure 5).

Changes in the video ethogram detected after surgery showed significantly ($P < 0.05$) increased summed passive behaviors at 2 h (Figure 4 C), specifically with significant ($P < 0.05$) increases in eyes closed or squinting at 48 h and in piloerection, weight shifting, and subtle body movement at 2 h (Figure 5). Summed active behaviors did not differ between anesthesia-analgesia and surgery conditions (Figure 4 F), but the individual active behavior forward or backward movement at 48 h was significantly ($P < 0.05$) decreased after surgery (Figure 5).

There were no differences between baseline and anesthesia–analgesia conditions (Figure 6 C) regarding electronic von Frey assessment, but mechanical hypersensitivity was significantly ($P < 0.01$) increased at 2 h after surgery compared with the anesthesia-analgesia condition.

Comparison and evaluation of postoperative pain assessments. To isolate changes associated with pain, we compared differences between the surgery and anesthesia–analgesia conditions. When the difference between these 2 conditions was 0, we surmised that pain was controlled effectively. After surgery, summed passive and active video ethogram behaviors revealed changes associated with pain until 8 h, whereas individual ethogram behaviors detected pain throughout most of the 96 h time points with the exception of 24 and 72 h. In addition, electronic von Frey testing detected changes associated with pain throughout the 96 h postsurgical period, except that no pain was detected in any of the treatment groups at 72 h.

The guinea pigs displayed each of the 5 cageside behaviors in the ethogram (eyes closed or squinting more than 50%, piloerection of more than 50% of hair coat, weight shifting, subtle body movement, and coprophagy) during the 3 conditions (baseline, anesthesia–analgesia, surgery). Scores obtained did not differ statistically between conditions or analgesic treatments (data not shown). Comparison of the cageside and equivalent video ethogram scores during the surgery condition revealed that cageside ethogram scores were significantly ($P < 0.0001$) lower than the video ethogram scores at all time points (Figure 8).

The time-to-consumption assessment revealed that approximately 30% to 60% of the guinea pigs were able to learn and perform the task of jumping onto the platform and chewing open the cardboard tube to reach a cache of hay across the different time points at baseline, but their performance was variable. Overall, there was no significant difference in performance of this task between conditions or treatment groups (data not shown).

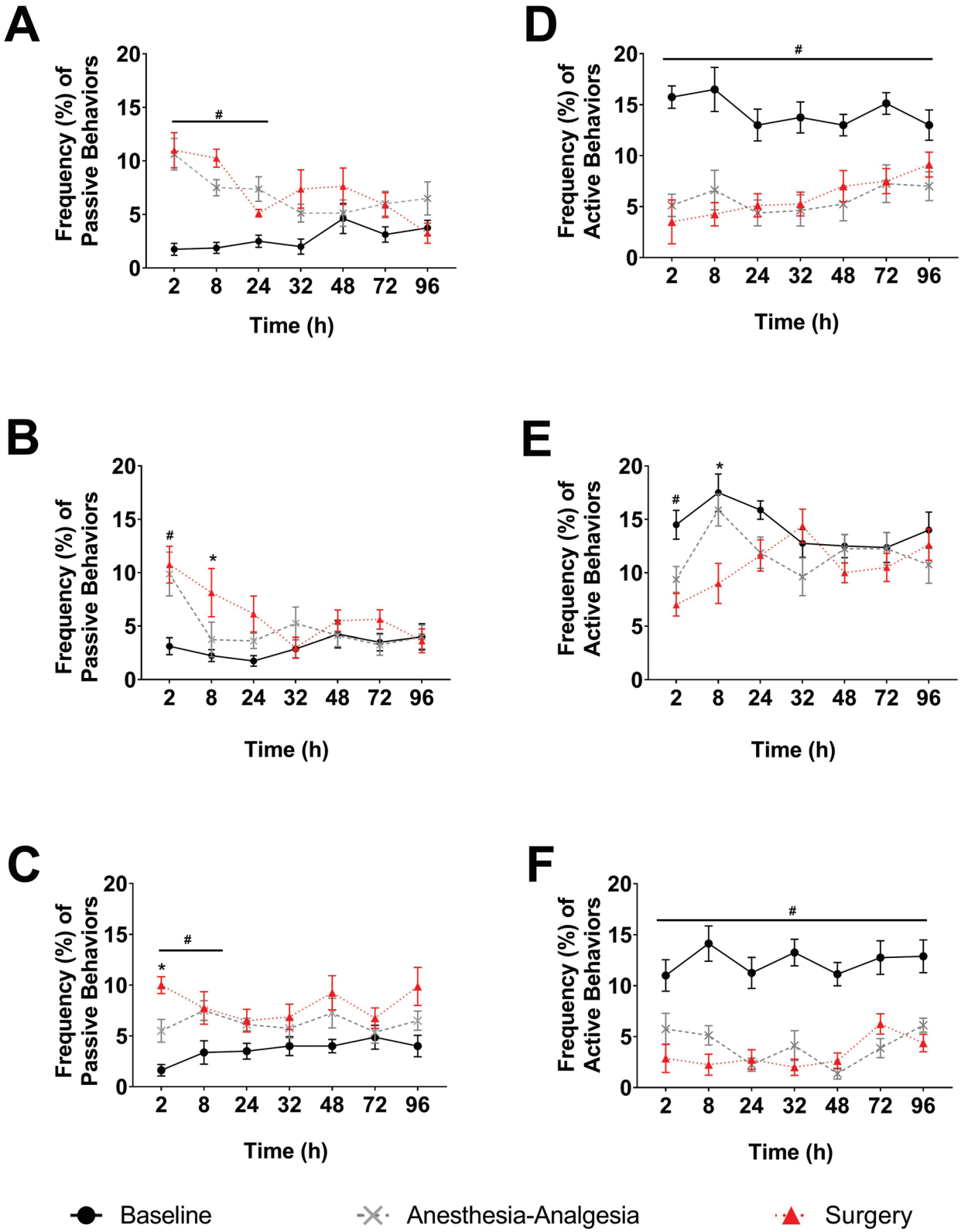

Figure 4. Frequency (mean ± SEM) of summed (A through C) passive and (D through F) active behaviors during baseline, anesthesia–analgesia, and surgery according to video ethogram assessment. (A and D) ER buprenorphine ($n = 8$), (B and E) carprofen ($n = 8$), and (C and F) multimodal ($n = 8$) groups. *, Value differs ($P < 0.05$) between anesthesia-analgesia and surgery conditions, indicating pain; #, value differs ($P < 0.05$) between baseline and anesthesia–analgesia conditions, indicating drug-associated effects.

Discussion

Guinea pigs are one of the most frequently used Animal Welfare Act–protected species for research anticipated to induce more than slight or momentary pain.[55] However, few studies have evaluated the clinical efficacy of the analgesics used most frequently to alleviate pain in guinea pigs. We therefore per-

	Time points (h)													
	Anesthesia–analgesia compared with baseline							Surgery compared with anesthesia–analgesia						
	2	8	24	32	48	72	96	2	8	24	32	48	72	96
Passive behaviors														
Eyes closed or squinting	C						M					M		
Piloerection	B C M	B M	B					M	C					
Weight shifting								M						
Subtle body movement	B	B M	B M	B M		B		B M						B
Incomplete movement					M						B			
Active behaviors														
Forward or backward movement	B	B M	C	B	M		M		C		C	M		
Body turn				B										B
Head or neck movement	B C M	B M	B M	B M	B M	B M	B M		C					
Rearing	B	B M												
Coprophagy		M	B M	M	B M	B M		C						

Figure 5. Summary of significant ($P < 0.05$) individual ethogram behaviors indicative of sedation (increase in passive and decrease in active behaviors during anesthesia-analgesia compared with baseline condition) and pain (increase in passive and decrease in active behaviors during surgery compared with anesthesia-analgesia) in ER buprenorphine-treated (B), carprofen-treated (C), and multimodal-treated (M) guinea pigs.

formed nonevoked and evoked pain measurements in guinea pigs to gain a more robust picture of the intensity and duration of pain that occurs postoperatively in a hysterectomy surgery model and evaluated analgesic efficacy of common pain relieving drugs. Specifically, we used a standard clinical assessment (body weight loss), 3 nonevoked assessments (cageside ethogram, video ethogram, and time-to-consumption test), and an established evoked (electronic von Frey) measure of pain under baseline, anesthesia–analgesia, and surgery conditions.

Multimodal analgesia provided the best analgesic efficacy to guinea pigs across multiple assessments of pain and nociception. Guinea pigs receiving multimodal analgesia demonstrated the shortest duration of ineffective pain coverage according to both video ethogram and von Frey assessments. The multimodal analgesia group demonstrated only a single time point—2 h after surgery—when pain was detected by both video ethogram and von Frey testing. The lack of analgesia at this time point might be due to a delay in achieving the therapeutic threshold of ER buprenorphine, as indicated in our pharmacokinetic data. However, pain was not detected by either video ethogram or von Frey testing at 2 h after surgery when ER buprenorphine was provided as a single analgesic agent. To ensure that buprenorphine has reached a therapeutic threshold prior to the onset of a painful stimulus, we recommend dosing animals 8 to 12 h before surgery. The ER formulation we used remained above the therapeutic threshold throughout the 96-h study period, thus demonstrating excellent analgesic coverage. The pain relief offered by other formulations evaluated in the literature have lasted only 12 to 24 h in mice,[10,29] as long as 72 h in rats,[19] and for 26 h in guinea pigs.[52] We were unable to find efficacy data regarding this multimodal combination in other rodent surgery models, but combinations of buprenorphine and meloxicam in rabbits after vascular cut-down procedures have been reported to minimize elevations in fecal corticosterone metabolites and promote weight gain postsurgery.[21] Similarly, multimodal combinations of carprofen and tramadol have reduced pain in rats after thoracic surgery more effectively than administering these analgesics alone.[9] Given our findings, we recommend multimodal treatment for guinea pigs undergoing hysterectomy or other surgeries of similar invasiveness.

The ER-buprenorphine group showed promising results also, but there were some indications that its pain relief was not as complete as for the multimodal group. Specifically, mechanical threshold data showed 2 time points of increased hypersensitivity, during which the drug was at the presumed therapeutic level of 1000 pg/mL. The efficacy data for other sustained-release buprenorphine formulations indicated sufficient coverage in other rodent postoperative models such as laparotomy in female CD1 mice[30] and tibial defect surgery in rats.[19]

Despite the compelling data supporting the analgesic efficacy of ER buprenorphine, several side effects should be considered when deciding to use this analgesic. When provided alone or in a multimodal combination, ER buprenorphine led to significant weight loss and behavioral sedation. Weight loss exceeding 10% in ER buprenorphine-treated guinea pigs both with and without surgical stimulus, as well as an inability to return to baseline weights within the 96-h study period, was concerning. This degree of weight loss could pose a problem when combined with experimental procedures that contribute to weight loss. In such cases, animals risk prematurely meeting experimental endpoints for weight loss, leading to their removal from study. ER buprenorphine–treated guinea pigs also displayed a significant alteration in their behaviors independent of surgical stimulus, demonstrating sedation through 96 h after administration. This finding could have important implications for studies using behavioral measures and highlights the need to evaluate analgesics not only at baseline and after surgery, but also after anesthesia and analgesia without surgery. By comparing data collected at baseline with those after anesthesia–analgesia, we were able to quantify and control for the drug effects on the behavioral ethogram. Therefore, when significant sedation occurred after anesthesia–analgesia alone as well as after surgery, we determined that the increase in passive behaviors and decrease in active behaviors after surgery were likely due to a sedative effect from the ER buprenorphine and not the result of unalleviated postoperative pain.

The carprofen regimen we used provided insufficient postoperative analgesic coverage. Carprofen-treated animals displayed the most time points indicative of hypersensitivity or pain according to von Frey testing, followed by video ethogram and body weight assessments, respectively. Although the carprofen group showed mild weight loss independent of the surgical condition, animals had recovered to baseline weights by 48 to 96 h postoperatively. In future studies, we will assess whether

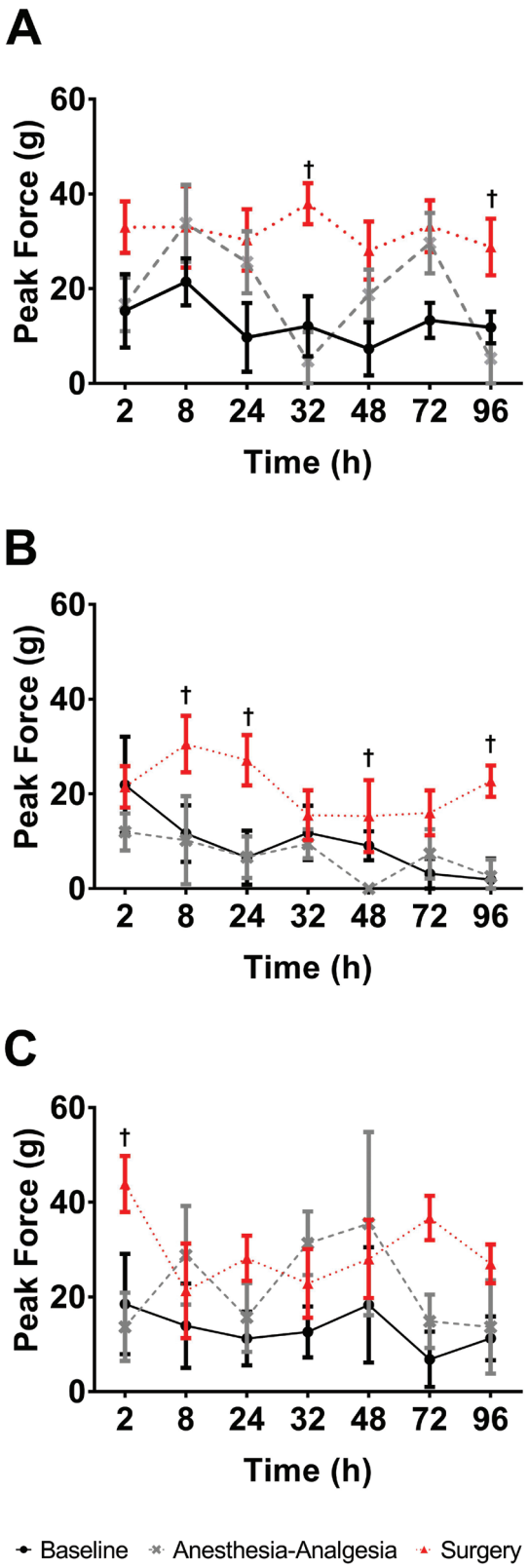

Figure 6. Peak force (g; mean ± SEM) calculated by the difference of mean electronic caudal von Frey measurement (adjacent to the incision and presumed to be painful) from the cranial measurement (presumed to be nonpainful) during baseline, anesthesia–analgesia, and surgery conditions. (A) ER buprenorphine, (B) Carprofen, (C) Multimodal treatment groups. †, Value differs ($P < 0.01$) between anesthesia–analgesia and surgery conditions, indicating pain.

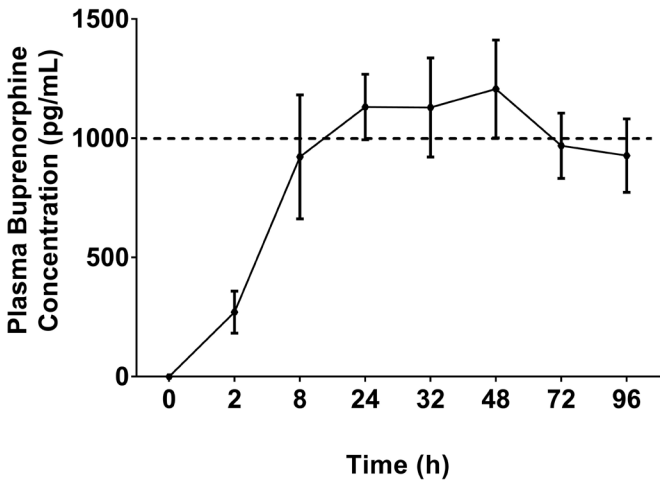

Figure 7. Plasma ER buprenorphine concentration ($n = 4$; mean ± SEM). The dashed line indicates the hypothesized therapeutic threshold (1000 pg/mL), based on values from human medicine.[16]

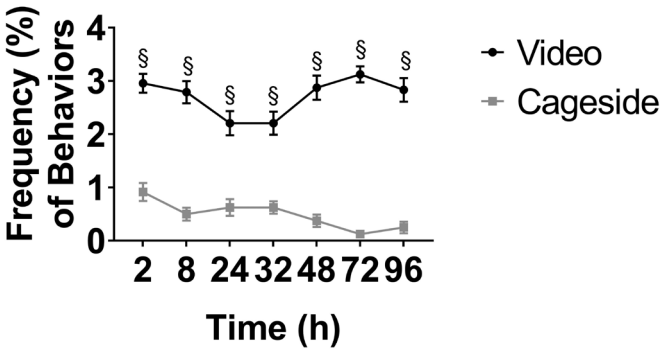

Figure 8. Frequency (mean ± SEM) of behaviors (the sum of eyes closed or squinting, piloerection, subtle body movement, weight shift, and coprophagy) determined through video compared with cageside ethogram pain assessment after surgery (§, $P < 0.0001$).

higher doses of this drug provide better analgesic coverage without exacerbation of the side effect of weight loss. Other studies have found carprofen to be effective in the early postoperative period[50] and as long as 4 h[49,51] after laparotomy in rats, whereas in mice, current doses did not significantly differ from saline groups,[1,40] and efficacy required much higher doses than those currently recommended for postoperative animals.[40] In addition, an anesthesia-only group might be useful, to evaluate any effects isoflurane might have on weight loss.

A limitation in our evaluation of analgesic efficacy was the lack of a negative-control group (that is, no-analgesia surgical group). This control was omitted because previous work in our lab[4,14] included this group in the initial validation of the video ethogram with the von Frey postoperative pain assessment in both guinea pig hysterectomy and castration surgical models. Therefore, in an effort to reduce animal numbers, we did not include a no-analgesia surgical group in the current study.

The secondary goal of our research was to compare the use of nonevoked and evoked measures of pain in a guinea pig postsurgical model of pain. The video ethogram assessment was previously validated by our lab for sensitively identifying the presence of pain in guinea pigs at 2 and 8 h after castration.[14] The ethogram used in the current study was modified from the original to classify the actions as (1) passive behaviors that are associated with pain and that increase in frequency during pain or (2) active behaviors that are not associated with pain and

that decrease in frequency during pain. Once categorized, the behaviors were analyzed both individually as well as summed in their category. Other behaviors including grooming, chewing, exploring, lying down, and shaking were evaluated, but differences between conditions were nonsignificant or the behaviors were not performed sufficiently frequently to contribute to the pain assessment score (data not shown).

We found that, individually, passive behaviors such as eyes closed or squinting, subtle body movement, and incomplete movement revealed pain at different time points throughout the 96-h observation period, but most behaviors identified pain primarily at 2 and 8 h after surgery. Similarly, the detection of pain by using individual and summed active behaviors most frequently occurred during the first 8 h after surgery. Collectively, these findings show that the modified ethogram is most sensitive for detecting pain at early time points, similar to the previous video ethogram results.[14] The difference in the time course of pain detection between individual and summed behaviors can likely be attributed to these behaviors being nonspecific. Therefore to better compile the changes that occur and are associated with pain, the monitored behaviors need to be combined to minimize any irrelevant changes that may not be important to the global pain picture. A potential limitation to video ethogram assessment is that the video clips were scored by a single blinded observer. In future studies, using multiple blinded observers would be valuable, to assess interobserver agreement.

The von Frey assessment was able to detect sensitivities to hypersensitivity throughout the 96-h study period. This finding differs from our previous work, in which von Frey testing detected significant hypersensitivity in guinea pigs until 8 h postoperatively only.[14] The previous work, however, used a surgical castration model in male guinea pigs, which arguably is a less invasive procedure than is hysterectomy and perhaps produced a less intense pain stimulus. In addition, the prior study was conducted in male guinea pigs, and male and female guinea pigs might differ in their pain responses, as has been demonstrated in other rodents.[42]

The difference in time course of pain detection between the von Frey and summed video ethogram assessments suggests that these tests measure different aspects of nociception. The von Frey assessment measures sensitivity to an evoked insult at the surgery site, whereas the video ethogram assessment reflects the response to the internal stimulus of spontaneous pain. The aspects of pain these 2 tests evaluate and any correlation they have requires further exploration. Similar to our results, other studies[11,13,28,46] have found an extended time course of hypersensitivity beyond measurements of pain from spontaneous measures. For example, the duration of mechanical hypersensitivity reportedly lasts beyond the pain detected from the rat grimace scale[13] and wheel-running activity[46] in inflammatory models of pain. Estimates of pain duration after surgery often vary depending on the test performed. Spontaneous pain behaviors in rats have been detected for 2 to 7 h after surgery,[49,51] whereas mechanical hypersensitivity after an incision may last as long as 1 wk.[6,47,58] Clinical indicators of pain in rodents, such as food consumption[1] and body weight,[1,5,51] have been altered for 1 to 2 d after surgery. Although in our case the video ethogram captured pain only during early time points, this tool might more accurately reflect the effects of pain on daily life and capture improvements in physical functioning, an important parameter in human pain treatment,[24] making video ethogram evaluation a more translatable pain assessment tool than evoked assessments.

However, both the von Frey and video ethogram assessments require specialized equipment, thus rendering them impractical for real-time cageside use. In contrast, typical cageside observation by animal care and research staff also brings challenges in the form of false-negative reports that animals are comfortable, due to their stoic nature as a prey species that typically masks pain, subsequently leading to the inappropriate use or omission of analgesics.[23] Therefore, we designed and evaluated the sensitivity of novel assessments with the goal of creating practical, easy to use, point-of-care pain assessments. The cageside ethogram assessment used some of the video ethogram behaviors, rendering the cageside version one that could be used quickly, cageside, and in real time. For this assessment, we selected behaviors that were easily observable, regularly performed, and had been determined to change in frequency during a state of pain. Our cageside ethogram results did not reveal any differences between baseline, anesthesia–analgesia, or surgery conditions in individual animals, indicating this test is not sufficiently sensitive to detect postsurgical pain. Given that the change in frequency of these behaviors among the 3 conditions was significantly increased after surgery when scored by video, which was recorded when no one was present in the room, we suspect that the insensitivity of the cageside test is largely due to the impressive ability of guinea pigs to suppress pain behaviors when observers are present. The inability to detect pain behavior on cageside exam underscores the difficulty research, husbandry, and veterinary staff face when assessing whether postoperative guinea pigs require additional pain medication and highlights why formal analgesia efficacy studies are necessary to provide evidence-based recommendations for dose and duration of analgesia treatment after surgery. The literature contains 2 studies evaluating differences between retrospective and real-time tests. One study found that baseline mouse grimace scale scores were higher when based on still images than when performed cageside.[41] The other found the rat grimace scale was comparable whether used in real-time or from still images.[32] Further work needs to be performed to identify cageside assessments that accommodate real-time scoring.

Similarly, we developed the time-to-consumption test to provide a simple cageside test that could be used for rapid pain assessment. Comparable to the time-to-integrate-to-nest test in mice,[48] the time-to-consumption test measured a practical species-specific behavior associated with fitness and survival during a state of pain. We designed this test to capture the motivational drive of an animal to perform a task to get a food reward. Previously our lab had struggled to identify a task that was sufficiently challenging to influence the motivation of the animals to perform it. For example, in pilot experiments, guinea pigs were so motivated to consume fresh parsley, they would do so with equal speed at baseline and soon after surgery. With the current iteration of this test, we increased the difficulty of acquiring a food treat by requiring the guinea pig to traverse a platform and actively chew or manipulate an object to retrieve hay, which we perceived to be a medium-value food reward. In this way, we hoped to increase the difficulty of the task and decrease the value of the reward to increase the cost-to-benefit ratio informing the choice the guinea pigs would make before and after surgery. Unfortunately, these changes did not influence the animals' motivation to perform the task under the 3 different conditions, and the test was therefore not sufficiently sensitive to detect postoperative pain. At this point, it likely would be beneficial to explore the use of other intrinsic behaviors that might be variably altered during a state of pain.

Other clinical indicators of pain, such as body weight loss, have been used previously to evaluate postoperative pain.[1,5,56] Our findings show that body weight loss is not an accurate proxy of pain with the analgesics assessed. The ER buprenorphine, multimodal, and carprofen treatments all resulted in significant reductions to body weight independent of a painful condition (that is, surgery). As mentioned earlier, it may be useful to investigate the effect of weight loss after isoflurane anesthesia alone to determine whether it contributes to this change. A study dosing rats with buprenorphine in the absence of a surgical stimulus found similar reductions to body weight.[5] Although our buprenorphine-treated guinea pigs lost weight equally after both the anesthesia–analgesia and surgery conditions, our carprofen-treated animals unexpectedly lost significantly more weight after surgery than after anesthesia–analgesia, implicating postoperative pain as a cause for that weight loss. Other studies in rats have reported that buprenorphine minimizes postoperative weight loss.[33-36] In contrast to our findings, carprofen has been reported to minimize weight loss after laparotomy in rats,[18] but the combination of dosing carprofen with buprenorphine for 24 h significantly decreased body weight in postoperative sham mice, which required as long as 72 h to return to weights similar to those of their analgesic controls.[1] We suspect that the weight loss in our ER buprenorphine and multimodal animals is related to an overestimated dose of buprenorphine. The formulation of ER buprenorphine we administered had not been studied previously in guinea pigs, and we therefore used allometric scaling to determine a dose. In a similar study using sustained-release buprenorphine in rats, the highest dose of the drug resulted in a 10% body weight loss compared with baseline weights.[8] In a future study, we will evaluate lower doses of ER buprenorphine to determine whether analgesic efficacy can be maintained in the absence of significant weight loss.

In summary, our findings from nonevoked and evoked pain assessments show that multimodal treatment provided the best analgesic coverage postoperatively in guinea pigs and that (according to our pharmacokinetic results) ER buprenorphine should be administered 8 to 12 h prior to surgery. Minimizing weight loss and sedation with this treatment requires further evaluation of lower dosages of ER buprenorphine and should be considered when selecting postoperative analgesics. In addition, our results show that using a combination of nonevoked and evoked measurements can provide an accurate and thorough pain assessment, but nonevoked measures likely provide a more clinically relevant picture. In contrast, common clinical assessments of pain, such as body weight measurements and cageside evaluations, may not be reliable indicators in guinea pigs.

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