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Comparison of Different Formulations of Extended-Release Buprenorphine in Perioperative Pain Management in Common Marmosets (*Callithrix jacchus*)

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Common marmosets (*Callithrix jacchus*) are increasingly used in biomedical research and often undergo surgery as part of IACUC-approved protocols. Therefore, pain control is essential to their clinical management and welfare. Extended-release buprenorphine is a valuable opioid analgesic option, as it can maintain plasma concentrations above therapeutic levels (0.1 ng/mL) for at least 72 h. However, no validated model is available to verify the analgesic effect of buprenorphine in marmosets. Therefore, this study compared the effects of buprenorphine-ER-LAB (Bup-ER-LAB) at 0.15 mg/kg and Ethiqa XR (EXR) at 0.15 and 0.1 mg/kg administered subcutaneously in marmosets undergoing surgical oocyte collection (*n* = 12 females) or vasectomy (*n* = 9 males). We hypothesized that these doses and formulations would provide similar analgesia during the 72-h postoperative period, determined with a marmoset composite measure pain scale designed for cage-side semiquantitative assessment of postoperative pain. The composite measure pain scale focused on animal appearance, activity, body posture/integument, respiration, surgical site, and social interactions, each with different subcategories and corresponding points to obtain a cumulative score. Animals were also assessed cage-side for locomotor activity and injection site reactions. No to minimal pain was scored, and no marmoset needed rescue analgesia. In total, 56% of the males and 25% of the females showed increased activity, which could last up to 48 h. Increased activity occurred in 57% of the BUP-ER-LAB group, 43% of the EXR 0.15 mg/kg group, and 14% of the EXR 0.1 mg/kg group (3 males and 4 females per group). Injection site reactions (erythema and/or swelling) occurred in 57% of the Bup-ER-LAB group, 29% of the EXR 0.15 mg/kg group, and 14% of the EXR 0.1 mg/kg group. Based on our results, we conclude that these formulations provide similar postoperative analgesia in marmosets, and an EXR dosage between 0.1 to 0.15 mg/kg provides adequate analgesia with less increase in activity and risk for injection site reaction.

Abbreviations and Acronyms: Bup-ER-LAB, buprenorphine-ER-LAB; CMPS, composite measure pain scale; EXR, Ethiqa XR; MIT, Massachusetts Institute of Technology; NHP, nonhuman primate; OPU, oocyte pickup

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Introduction

The common marmoset (*Callithrix jacchus*) has emerged as a valuable biomedical research model due to its relatively small size, rapid reproductive rate among NHP species, and physiologic similarities to humans.[1,2] Importantly, transgenic marmosets have been successfully generated via assisted reproduction technologies as a model for neuroscience and neurodegenerative diseases.[3–5] Surgical manipulations are often described in IACUC-approved protocols involving marmosets, which necessitate careful consideration of pain management strategies to ensure appropriate animal wellbeing and validity of experimental outcomes. However, there are limited evidence-based analgesic evaluations for marmosets. To date, there are no studies that evaluate the pain-relieving ability of analgesics for this species.

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Buprenorphine, a synthetic opioid derivative with potent analgesic properties, is often administered to alleviate pain in marmosets and other NHPs.[6–8] The development of extended-release formulations of buprenorphine has garnered significant attention in veterinary medicine, including marmosets.[9,10] One formulation is a compounded product called buprenorphine-ER-LAB (Bup-ER-LAB, formerly known as buprenorphine SR-LAB).[10]

A single subcutaneous dose of Bup-ER-LAB at 0.2 mg/kg in marmosets resulted in mean drug concentrations exceeding the assumed therapeutic threshold (0.1 ng/mL) for ~3.5 d. The other formulation is Ethiqa XR (EXR), which is pharmaceutical grade and even FDA-indexed for NHPs. Pharmacokinetic and safety studies of EXR have been conducted in marmosets[9] and cynomolgus macaques.[11] Comparisons of Bup-ER-LAB and EXR in marmosets have demonstrated that Bup-ER-LAB at 0.15 mg/kg, EXR at 0.15 mg/kg, and EXR at 0.1 mg/kg SC have similar plasma concentration–time curves, having exceeded 0.1 ng/mL for ~3 d after a single dose.[9] However, the therapeutic plasma buprenorphine concentration threshold has not yet been determined for marmosets. In humans, the therapeutic plasma

concentration range of buprenorphine is 0.1 to 0.5 ng/mL based on correlations between pharmacokinetic studies and clinical assessment of subjects with postoperative or chronic pain and analgesiometric tests,[12–14] and similar therapeutic plasma concentration ranges for buprenorphine are considered for pain management in NHPs.

Currently, no validated methods are available to assess pain or analgesic efficacy in marmosets. Therefore, we compared the effects of Bup-ER-LAB and EXR as part of a multimodal analgesic regimen in female marmosets undergoing oocyte harvest (oocyte pickup [OPU]), and males undergoing vasectomy in a transgenic program. A marmoset composite measure pain scale (CMPS) was designed for cage-side semiquantitative assessment of postoperative pain to measure analgesic efficacy. We hypothesized that a single subcutaneous dose of EXR at 0.1 mg/kg provides parallel analgesia and yields similar pain scoring when compared with EXR at 0.15 mg/kg and Bup-ER-LAB at 0.15 mg/kg.

Materials and Methods

Animals. Adult common marmosets were selected for OPU or vasectomy based on the needs of the transgenic program at the Massachusetts Institute of Technology (MIT) and Broad Institute (Cambridge, MA). Animals were determined to be healthy by a veterinarian based on complete physical, hematologic, and biochemical examinations (total $n = 21$). Marmosets were divided into 3 treatment groups: EXR 0.1 mg/kg (3 males, 3 to 6 y old; 4 females, 3 to 5 y old), EXR 0.15 mg/kg (3 males, 2 to 6 y old; 4 females, 2 to 5 y old), and Bup-ER-LAB 0.15 mg/kg (3 males, 2 to 3 y old; 4 females, 3 to 6 y old) (Figure 1). Two females in the EXR 0.1 mg/kg group, one female in the EXR 0.15 mg/kg group, and 3 females in the Bup-ER-LAB 0.15 mg/kg group had one previous OPU before being assigned to this study. There was a minimum 3-mo rest time between the first and second OPU. The remaining females in this study had undergone their initial OPU. Marmosets were assigned to their respective treatment groups using simple randomization.

All animals were >300 g in body weight and had a body condition score ranging from 2 to 2.52 to 3.5 on a scale of 1 to 5.[15] They were socially housed, in either a pair or family group, in enriched Britz cages (30 × 32 × 67 in. [76 × 81 × 170 cm]). Enclosures contained perches, nest boxes, hammocks, manzanita wood branches, hanging toys, and ferret balls. Housing rooms were maintained at 78.0 ± 2.0 °F (23.3 ± 1.0 °C) with a relative humidity of 30% to 70%. Full spectrum lighting was provided on a 12-h light/12-h dark cycle. The diet consisted of extruded biscuits (Teklad New World Primate diet 8794, Mazuri 5L7L 50/50 blend, Envigo, Madison, WI, and LabDiet New World Primate 5040, Purina, St. Louis, MO) soaked lightly in water, gel diet (Mazuri callitrichid gel diet no. 5B34), fruits and vegetables, and various protein sources such as eggs, yogurt, cottage cheese, and beans. Chlorinated reverse osmosis–purified water was provided ad libitum.

All study procedures were performed under approval from the MIT Committee on Animal Care and the Broad Institute's IACUC and followed all applicable federal, state, and local guidelines and regulations. MIT and the Broad Institute are both AAALAC accredited.

Sedation, anesthesia, and surgical preparation. The induction drugs were tailored to each individual animal and included alfaxalone (10 mg/mL, Jurox, Rutherford, NSW, Australia), midazolam (5 mg/mL, Akorn, Lake Forest, IL), and atropine sulfate (0.54 mg/mL, VetOne, Pomona, CA). A comprehensive summary of drugs and dosages is shown in Table 1. After aseptic skin preparation,[16] a local anesthetic block was applied to the planned incision site using lidocaine (2%, Phoenix, St. Joseph, MO), bupivacaine (0.5%, Hospira, Lake Forest, IL), or a combination of both. For all procedures, an angiocatheter was placed in a peripheral vein (saphenous, cephalic, or tail) prior to surgery. Isoflurane (1.0% to 3.0%, Dechra, Overland Park, KS, in 100% oxygen flow 0.8 to 1 L/min) was delivered via a face mask or an appropriately sized endotracheal tube for maintenance of general anesthesia. In addition to isoflurane, some marmosets also received an additional dose of alfaxalone, ketamine (100 mg/mL, Dechra, Overland Park, KS) or lidocaine splash block, and all females received a single dose of cefazolin (1 g, Hikma, Terrugem, Portugal). Intraoperative monitoring included heart rate, respiratory rate, body temperature, and pulse oximetry. For recovery, animals that had been given midazolam were reversed with flumazenil (0.1 mg/mL, Hikma, Terrugem, Portugal). Most females also received a dose of maropitant (10 mg/mL, Cerenia, Zoetis, Kalamazoo, MI) as a preemptive antiemetic.

Surgeries. The OPU and vasectomy procedures were performed by one surgeon who was blinded to the tested drug treatment groups and was not the same person who was the observer and scored the CMPS.

OPU. The female marmosets were induced with exogenous gonadotropins 25 to 50 IU intramuscularly or subcutaneously to obtain oocytes. The ovarian cycles were monitored by measuring the plasma progesterone level. Animals were elected for OPU surgery based on the desirable progesterone range. The skin of the anesthetized marmoset was prepped aseptically, a local block was administered, and a sterile drape was placed (see Sedation, anesthesia, and surgical preparation). A midline skin incision at the caudal abdomen was followed by an abdominal wall incision into the abdominal cavity. The ovaries were exteriorized individually, and the follicular surfaces were aspirated with a 25-gauge needle attached to a 3-mL syringe. Minimal hemorrhage after aspiration was controlled by applying gentle pressure with a damp sterile gauze square. If deemed necessary, a sterile cotton-tipped applicator soaked in saline was used to apply a scant amount of diluted epinephrine to ovarian

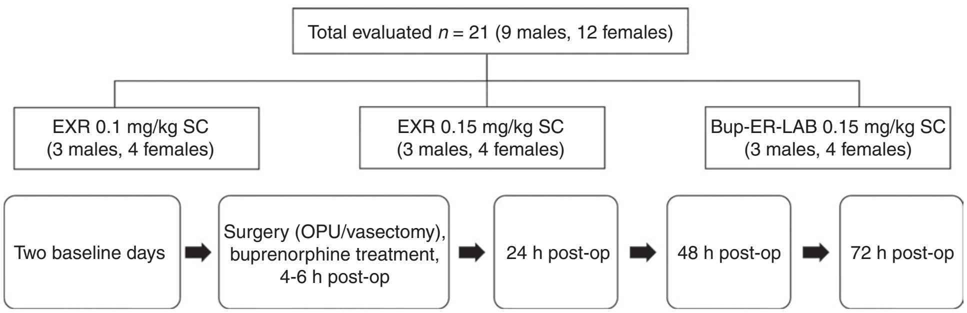

Figure 1. Graphical timeline of experimental methods and design. Each marmoset was assessed on 2 baseline days within 72 h before surgery to assess behavior, activity level, and pain assessment. Subsequent evaluations were performed at 4 to 6, 24, 48, and 72 h postoperatively.

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Table 1. Demographic, sedation protocol, perioperative, and postoperative medications

Animal ID	Sex	Weight (g)	Body condition score out of 5	Induction	Local block	Maropitant 1 mg/kg SC preemptive antiemetic	Adjunctive intraoperative drugs	Postoperative nonopioid drugs	Postoperative opioid drugs
M1	Male	486	3	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
M2	Male	463	3–3.5	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	None	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
M3	Male	344	2.5	Alfaxalone 5 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	No	None	Meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
F1	Female-first OPU	421	3	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
F2	Female-first OPU	379	2.5	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
F3	Female-second OPU	362	2.5–3	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
F4	Female-second OPU	411	2.5	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.1 mg/kg SC
M4	Male	392	3	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
M5	Male	444	3	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV, lidocaine 1 mg/kg splash block	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
M6	Male	342	2.5	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	None	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
F5	Female-first OPU	372	2.5	Alfaxalone 10 mg/kg IM	Bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV, ketamine 10 mg/kg IM, cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
F6	Female-first OPU	385	3	Alfaxalone 12 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
F7	Female-first OPU	353	2.5	Alfaxalone 6 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Atropine 0.02 mg/kg IM, meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
F8	Female-second OPU	442	2.5–3	Alfaxalone 12 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	EXR 0.15 mg/kg SC
M7	Male	454	3.5	Alfaxalone 8 mg/kg IM, midazolam 0.1 mg/kg IM	Bupivacaine 2 mg/kg	No	None	Meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC

(*continued*)

Table 1. (Continued)

Animal ID	Sex	Weight (g)	Body condition score out of 5	Induction	Local block	Maropitant 1 mg/kg SC preemptive antiemetic	Adjunctive intraoperative drugs	Postoperative nonopioid drugs	Postoperative opioid drugs
M8	Male	424	3	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC
M9	Male	384	3.5	Alfaxalone 8 mg/kg IM, midazolam 0.3 mg/kg IM	Lidocaine 2 mg/kg, bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC
F9	Female-first OPU	437	2.5	Alfaxalone 12 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC
F10	Female-second OPU	458	2.5	Alfaxalone 8 mg/kg IM, midazolam 0.1 mg/kg IM	Bupivacaine 2 mg/kg	No	Alfaxalone 2 mg/kg IV, cefazolin 20 mg/kg IV	Flumazenil 0.02 mg/kg IV, meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC
F11	Female-second OPU	360	2–2.5	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC
F12	Female-second OPU	517	3.5	Alfaxalone 12 mg/kg IM, atropine 0.02 mg/kg IM	Lidocaine 2 mg/kg	Yes	Cefazolin 20 mg/kg IV	Meloxicam 0.2 mg/kg SC	Bup-ER-LAB 0.15 mg/kg SC

tissue with minor hemorrhage (1 applicator per ovary). After aspiration and assurance of hemostasis, the abdomen was lavaged with ~30 mL of sterile warm 0.9% NaCl. The abdominal wall was closed with 4-0 absorbable monofilament in a simple continuous pattern, and the skin was closed with 5-0 absorbable monofilament suture in an interrupted pattern, and a light bandage was placed over the surgical site.

Vasectomy. The skin of the anesthetized marmoset was prepped aseptically, a local block was administered, and a sterile drape was placed (see Sedation, anesthesia, and surgical preparation). A midline skin incision at the level of the pubis was made. The subcutaneous tissue layer was bluntly dissected with a small hemostat to isolate the spermatic cord and then the vas deferens. The vasa deferentia were cauterized with a cautery pen. After hemostasis was assured, the subcutaneous tissue layer was closed with absorbable 5-0 monofilament in a continuous pattern, the skin was closed with an absorbable 5-0 monofilament suture in an interrupted pattern, and a light bandage was placed over the surgical site.

Postoperative pain management. Meloxicam (5 mg/mL, Pivetal, UK) at 0.2 mg/kg SC was administered once on the day of surgery by the end of the procedure, followed by oral meloxicam (1.5 mg/mL, Pivetal, UK) at 0.1 mg/kg once a day for the next 3 d.

A dose of extended-release buprenorphine was administered as soon as the animal was fully awake from anesthesia to avoid potential respiratory depression.[17] Marmosets were fasted overnight with free access to water. They were randomized to receive either a single dose of Bup-ER-LAB (1 mg/mL, ZooPharm, Laramie, WY) at 0.15 mg/kg SC using a 1-mL low dead space syringe and 22-gauge, ¾-in. needle, or EXR (1.3 mg/mL, Fidelis Animal Health, North Brunswick Township, NJ) at 0.1 mg/kg or 0.15 mg/kg SC using a 1-mL low dead space syringe and 27-gauge, ½-in. needle. The drug was administered along the abdomen for ease of monitoring injection sites. Buprenorphine injection sites were circled with a surgical skin marker immediately after administration to facilitate monitoring during the 72-h observation period.

CMPS. A marmoset CMPS was designed for cage-side semiquantitative assessment of postoperative pain, focusing on 6 categories: animal appearance, activity, body posture/integument, respiration, surgical site, and social interactions (with the human observer and their cage-mates) (Table 2). A previous study showed buprenorphine-induced hyperactivity.[9] Therefore, activity was assessed separately. Each item on the CMPS was assigned a numerical score from 0 to 3.

The score for each category was added to determine a total pain score for each time point. A total pain score of 0 corresponded to no pain, and a total score of 1 corresponded to minimal pain. No additional action outside of standard postsurgical monitoring was warranted for total scores of 0 or 1. A total pain score of 2 corresponded to mild pain, a score of 3 to 5 corresponded to moderate pain, and a score 6 or higher corresponded to severe pain. Any score of 2 or higher prompted further veterinary assessment and intervention, including rescue analgesia (for example, buprenorphine HCl).

Each marmoset had 2 baseline CMPS recordings (5 to 10 min) within 72 h before the surgery on different days (one in the afternoon and one in the morning) to assess the normal behavior, activity levels, and baseline pain assessment. Subsequent CMPS recordings were performed to determine a total pain score for each of the following time points: 4 to 6, 24, 48, and 72 h after surgery (a total of 6 recordings for each marmoset). Increased activity in this study was defined as the visually observed rise

Table 2. Components of the composite measure pain scale for common marmosets

	Pain score			
Category	0	1	2	3
Appearance	BAR	QAR	QDR	Recumbent
Activity	Moving normally in the cage	Decreased activity in the cage	Lack of interest in the environment	Immobile
Body posture and integument[a]	Normal posture	Mild piloerection Mildly (intermittent) hunched	Moderate piloerection Moderately hunched Intermittent trembling	Generalized piloerection Severely hunched Continuous trembling
Respiration[b]	Normal	Abnormal	—	—
Surgical site	No attention	Infrequent scratching without compromising the incision	Guarding	Biting
Social interaction[c]	Eating treats	Not eating treats	Aggression, isolation	—

BAR, bright, alert, responsive; QAR, quiet, alert, responsive; QDR, quiet, depressed, responsive.
[a]Hunch posture at any time, either resting or moving. Piloerection refers to the raising of hair.
[b]Abnormal refers to increased respiratory rate and/or effort.
[c]Interaction with the observer and the cage-mate(s). Eating refers to taking and eating a high-reward treat (marshmallow) when offered.

in locomotor activity, characterized by purposeless circling movements or back-and-forth jumps between 2 surfaces (for example, between the hammock and nest box) in the enclosure. Such movements had to occur at least twice during the CMPS observation period and lacked a clear goal, such as interacting with enrichment, engaging with a cage mate, or obtaining a food item. The activity level of each marmoset was scored as either normal (that is, normoactive) or increased (that is, hyperactive). Throughout the entire study, a veterinarian familiar with marmoset behavior who was blinded to the treatments served as the sole observer for all pain assessments using CMPS. Also, for each time point, the animal was given an acclimation time of 2 to 3 min, during which the observer stood in front of the animal's cage before the observer could start assessing the animal.

Adverse events scoring. Animals were also assessed for adverse events such as local drug injection site adverse events at each time point. An injection site reaction was defined as the presence of erythema and/or swelling observed at any time point up to 72 h after the buprenorphine injection. Injection site assessment was performed by the same veterinarian who scored the CMPS and was blinded to the treatments. All study animals were independently monitored twice daily on routine veterinary rounds by technicians and veterinary staff, separate from the study's blinded observer.

Statistical analysis. The 2 recorded CMPS baselines on 2 separate days were averaged for each animal. All subsequent (postoperative) CMPS scores for each animal were normalized by subtracting the averaged baseline score. The Friedman test was used to compare the normalized total CMPS scores of Bup-ER-LAB and EXR groups, as well as between males and females. Activity and adverse events were analyzed via a Fisher exact test to compare between Bup-ER-LAB and EXR groups, as well as between males and females. Statistical analyses were performed using GraphPad Prism version 10.1.2, and a P value of 0.05 or less was defined as significant. In addition, a power analysis was conducted with G*Power version 3.1.9.7 (effect size of 0.25, α error probability of 0.05, and a desired power of 0.95) to determine the necessary sample size.

Results

Excluded animals. Two females that underwent an OPU and received postoperative buprenorphine treatment were excluded from the study, one due to an inadvertent change in the administration schedule of another postoperative analgesic (meloxicam) (in the Bup-ER-LAB group) and the other due to a skin hypersensitivity reaction to the bandage adhesive material (in the EXR 0.1 mg/kg group). Also, one male was excluded due to dehiscence of the vasectomy incision (in the Bup-ER-LAB group). After excluding these 3 animals, 21 animals remained in this study, and each group consisted of 3 males and 4 females.

CMPS scores. All baseline CMPS scores were a 0. Postoperative CMPS scores for all study animals were either a 0 or 1 (Figure 2). In the EXR 0.1 mg/kg group (3 males and 4 females), one female (first-time OPU) was given a score of 1 at 48 h due to a mild hunched posture, and the rest of the animals had a total pain score of 0. In the EXR 0.15 mg/kg group (3 males and 4 females), one female (first-time OPU) was given a score of 1 at 4 to 6 h due to a mild hunched posture and again at 24 h due to mild piloerection. Another female (second-time OPU) was given a score of 1 at 4 to 6 h due to mild piloerection, and the rest of the animals had a total pain score of 0 in this group. In the Bup-ER-LAB 0.15 mg/kg group (3 males and 4 females), 2 females (both second-time OPU) were given a score of 1 at 4 to 6 h due to mild piloerection, and the rest of the animals had a total pain score of 0. No marmoset in this study needed rescue analgesia at any time point according to CMPS evaluations, nor were any additional analgesics needed based on veterinary assessments of animals independent of the study. In addition, no signs of aggression were observed or reported at any time. There were no statistically significant differences in total pain scores for marmosets undergoing surgical OPU or vasectomy and receiving either Bup-ER-LAB or EXR when comparing the drug groups or males compared with females ($P > 0.05$).

Drug-induced increase in activity. An increased activity up to 48 h after administration of either formulation of extended-release buprenorphine was occasionally observed in male and female marmosets. In total, 56% of males (5 out of a total of 9 males) and 25% of females (3 out of a total of 12 females) were hyperactive (Figure 3). Additionally, 14% of the EXR 0.1 mg/kg group, 43% of the EXR 0.15 mg/kg group, and 57% of the Bup-ER-LAB 0.15 mg/kg group exhibited increased activity ($n = 7$ for each group, 3 males and 4 females) (Figure 4). In the EXR 0.1 mg/kg group, only 33% of the males were more active, and all of the females were observed to have normal activity. In the EXR 0.15 mg/kg group, 67% of the males and 25% of the females were hyperactive. In the Bup-ER-LAB 0.15 mg/kg group, 67% of the males and 50% of the females were hyperactive ($n = 7$ for each group, 3 males and 4 females)

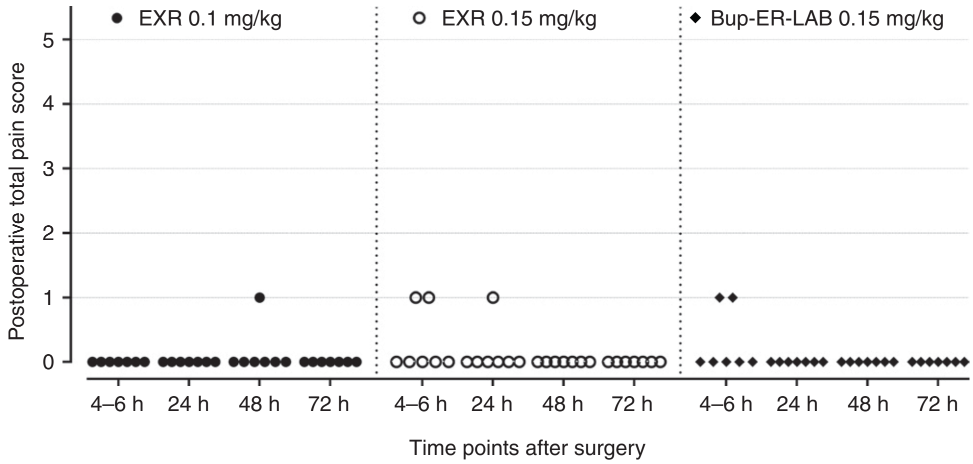

Figure 2. Scatter plot of normalized postoperative total pain scores. Total pain scores were determined by using the composite measure pain scale (CMPS) in common marmosets undergoing surgical oocyte collection (OPU) or vasectomy and receiving different formulations of extended-release buprenorphine. Each data point represents an individual marmoset's total pain score at various time points up to 72 h after surgery ($P > 0.05$).

(Figure 5). There were no statistically significant differences in increase in activity when comparing the drug groups or males compared with females ($P > 0.05$).

Adverse events. No animals had skin lesions at any of the baseline evaluations. Injection-site reaction after administration of either formulation of extended-release buprenorphine was observed. In total, 14% of the animals in the EXR 0.1 mg/kg group (1 female), 29% of the animals in the EXR 0.15 mg/kg group (1 male and 1 female), and 57% of the animals in the Bup-ER-LAB 0.15 mg/kg group (2 males and 3 females) had injection-site reactions ($n = 7$ for each group, 3 males and 4 females). (Figure 6). None of the injection-site reactions needed to be treated medically, and there were no statistically significant differences in the occurrence of injection-site reactions when comparing the drug groups or males compared with females ($P > 0.05$).

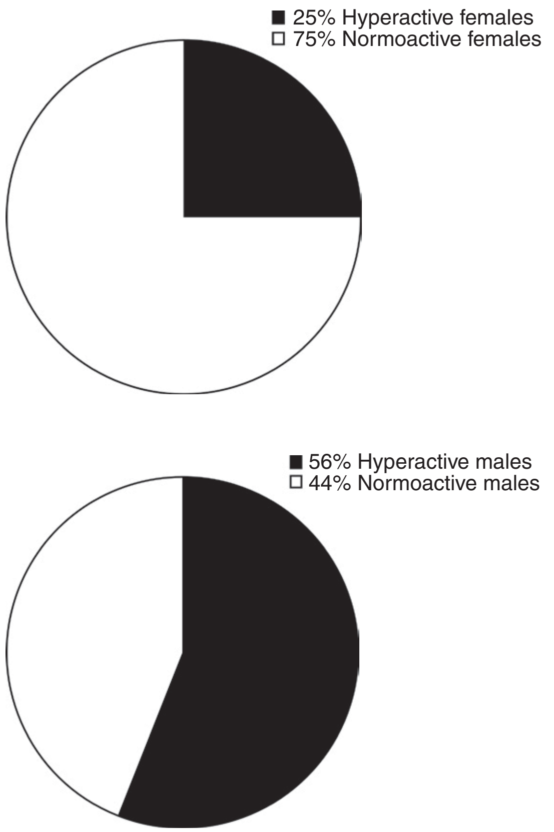

Figure 3. Hyperactivity after administration of different formulations of extended-release buprenorphine in male and female common marmosets. Hyperactivity could last up to 48 h after the buprenorphine injection ($P > 0.05$).

Discussion

Appropriate pain mitigation is a basic foundation of responsible animal research and is required by multiple animal research regulatory policies to ensure the ethical and humane treatment of NHPs. This involves promptly identifying pain and using multimodal analgesics to minimize pain and discomfort. This is essential for improving recovery, reducing complications, and enhancing overall patient outcomes and the reliability of research data. In this study, we compared the efficacy of 2 formulations of extended-release buprenorphine, Bup-ER-LAB (at 0.15 mg/kg SC) and EXR (at 0.1 and 0.15 mg/kg SC), as part of a multimodal analgesic regimen for common marmosets undergoing standard surgical procedures in our transgenic program at MIT and the Broad Institute. Efficacy was evaluated by developing and using a marmoset CMPS designed for cage-side semiquantitative assessment of postoperative pain. We demonstrated that both formulations of extended-release buprenorphine provide sufficient pain control in a multimodal analgesic regimen for common marmosets undergoing OPU and vasectomy. In addition, our data suggest that EXR at either dose, 0.1 and 0.15 mg/kg, may cause fewer incidences of observed increase in activity and fewer injection site reactions, although these findings were not statistically significant ($P > 0.05$).

Assessing pain in NHPs is essential for effective laboratory animal medicine and welfare. However, it is challenging due to animals' inability to communicate like humans. Behavioral and physiologic changes can sometimes indicate pain, although they may be subtle and not reliably recognized. Altered posture and movement, facial expression, vocalization, appetite, grooming, social behavior, heart rate, blood pressure, and respiratory rate may indicate pain.[18] Grimace scales based on changes in facial expression have been established for mice,[19] rats,[20] rabbits,[21] ferrets,[22] and piglets[23] to recognize pain. The University of Glasgow[24] and the University of Melbourne[25] have developed CMPS for acute pain in dogs.

Similar attempts have been made to establish a CMPS for different Old World NHP species.[26–28] A survey result from

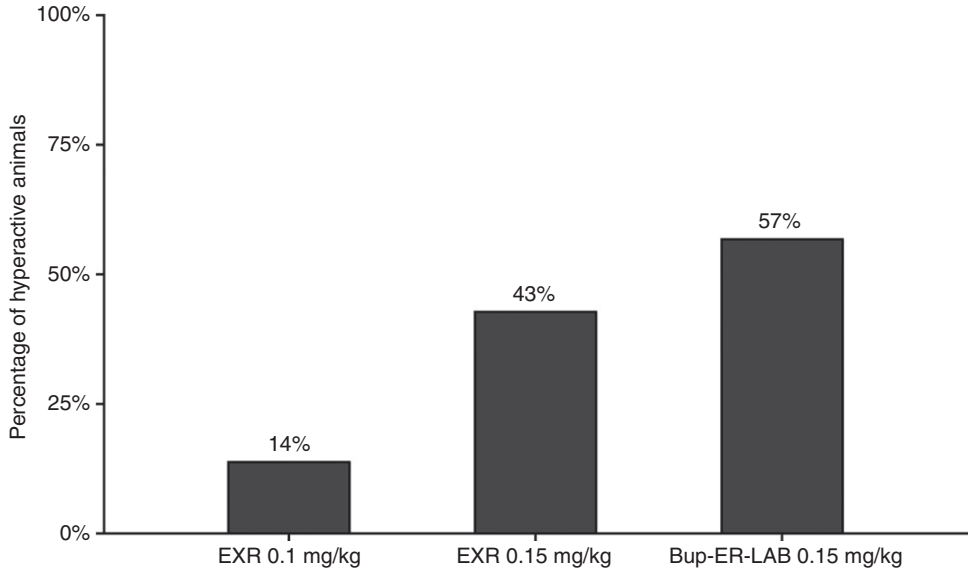

Figure 4. Percentage of hyperactive animals after administration of different formulations of extended-release buprenorphine in common marmosets (sexes combined) ($P > 0.05$).

veterinarians who work with Old World NHPs showed that the most important signs of pain in these animals are guarding the surgery/wound site, abnormal posture, reluctance to move, and restlessness, and the analgesic choices were nonsteroidal antiinflammatory meloxicam (then carprofen) and buprenorphine.[29] However, this survey did not separate the regular or extended-release formulation of buprenorphine, and it was mentioned as being grouped together. A similar survey on important signs of pain in New World NHPs has not been published to date. No CMPS for common marmosets has been reported in the literature so far, and no validated efficacy test is available to verify the analgesic effect of buprenorphine in this species. However, behavioral and physiologic changes, although subtle, may serve as signs of pain, as in other species.

Historically, the grimace scale, which focuses on facial expressions and postures specific to pain, has been used to measure pain semiquantitatively in different species.[8,19–23] These facial grimace scales were measured through direct (human observer) or indirect (video or photo capturing) cage-side observations. In a laboratory setting, prey species such as common marmosets may conceal their pain when human subjects are present, which can make it difficult to detect signs of pain.[28,30,31] In addition, adopting the grimace scale can be challenging in NHPs due to complex facial expressions and behaviors.[29] There are validated grimace scales for *Macaca mulatta*, *Macaca fascicularis*, and *Macaca fuscata* using methods such as the facial action coding system, geometric morphometrics, or video recording and photo capturing.[8,26,27] However, no such scales are available for common marmosets. This could be due to technical challenges to directly or indirectly capture their subtle and complex facial expressions, especially considering that they typically live in family groups.

Due to technical and practical limitations, setting up a video camera to indirectly record animals was not possible in this study. Therefore, a single veterinarian observer blinded to the treatment assignments performed cage-side assessments of the animals. One limitation of this study was that a single observer scored

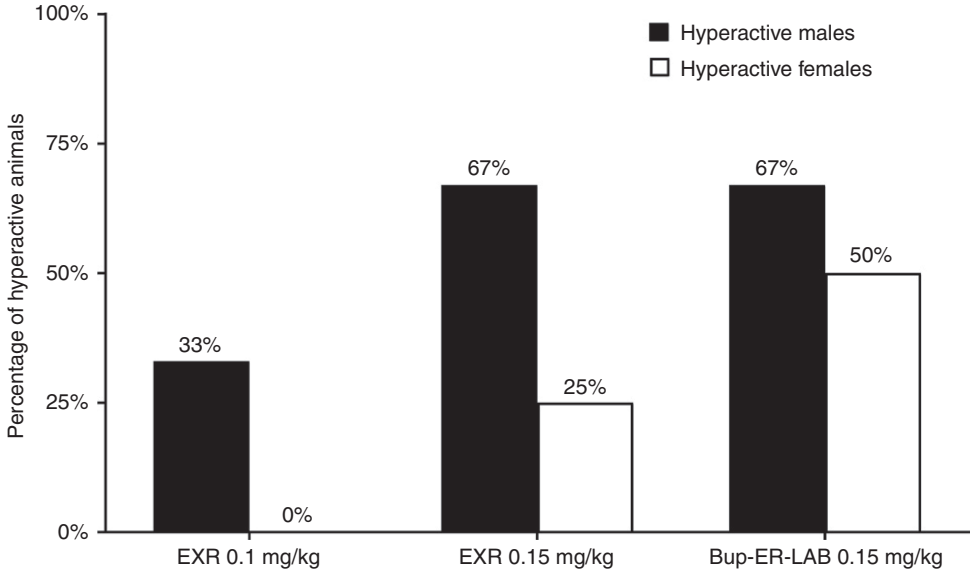

Figure 5. Percentage of hyperactive male and female common marmosets after administration of different formulations of extended-release buprenorphine ($P > 0.05$).

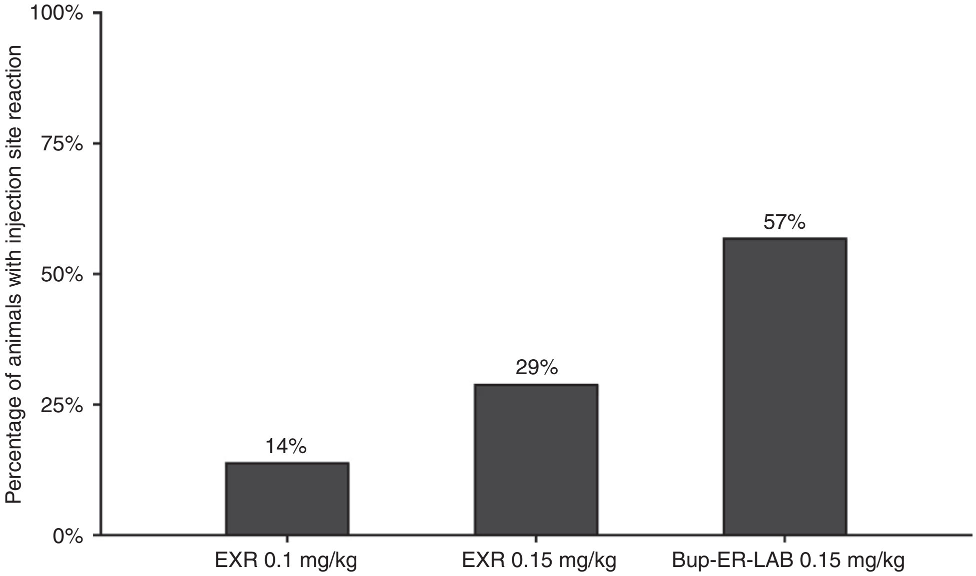

Figure 6. Percentage of animals with injection site reaction after administration of different formulations of extended-release buprenorphine in common marmosets (sexes combined). An injection site reaction was defined by observing the presence of erythema and/or swelling at any time point up to 72 h after the buprenorphine injection ($P > 0.05$).

all of the study animals. Future studies should aim to establish interobserver reliability to allow multiple users of the CMPS to assess marmosets, whether related to buprenorphine or for other applications. Future studies may also consider more complex animal behavior analyses via video recordings in scenarios when this setup is feasible. However, most clinical assessments of NHPs used in research are made cage-side. Thus, the practical application of the assessment tool must be considered.

Based on our CMPS analysis, there were no statistically significant differences in total pain scores for marmosets undergoing surgical OPU or vasectomy and receiving either Bup-ER-LAB or EXR when comparing the drug groups or males compared with females, and no marmoset in this study needed rescue analgesia at any time point ($P > 0.05$). All study animals were observed to have either minimal (total pain score = 1) or no (total pain score = 0) postoperative pain. Some females had pain scores of 1 at a few time points, but pain scores for all males at all time points were 0, indicating that some females may have had minimal pain at some time points, but males had less pain. In this study, a few females had one previous OPU surgery in their history. This could be a possible reason why females more frequently exhibit a pain score of 1, as prior surgeries can sometimes result in adhesions and fibrosis, leading to increased discomfort for future procedures. However, no statistical significance in total pain scores between males and females was found in this study ($P > 0.05$), but it is noteworthy that the sample size in the study was relatively small due to limited animals available for study enrollment.

Sex differences in pain and its relief arise from a complex interaction of genetic, anatomic, physiologic, neuronal, hormonal, psychologic, and social factors.[32] For example, in humans, females show more variability in their pain experiences than do males, with increased pain sensitivity and a higher prevalence of painful diseases. Similar to humans, NHP pain tolerance can be different between sexes due to hormonal differences, the endogenous opioid system, stress, coping mechanisms, and the social hierarchy system.[33] One possible explanation for the differences in pain scores between males and females in marmosets could be the variation in plasma concentration of buprenorphine. Fitz and colleagues showed that female marmosets had lower plasma concentrations when administered with extended-release buprenorphine compared with males during the 72-h postinjection period, while the study was underpowered.[10] In our study, although no animals needed rescue analgesia, it is possible that females overall had lower plasma buprenorphine concentrations compared with males. We did not collect blood for buprenorphine concentrations to avoid complications for the pain scoring process, as the stress of capturing the animal could interfere with pain tolerance. However, adequately powered studies in the future could compare plasma buprenorphine concentrations in males and females to validate if a sex difference exists. In addition to sex differences in pain tolerance and perception, the level of pain experienced after surgery can vary widely depending on the specific type of surgery performed. The other possible explanation for the differences between males and females in pain scores could be due to the differences in the OPU and vasectomy surgeries. The OPU procedure is more invasive than a vasectomy in marmosets, as it involves opening the abdominal cavity and aspirating the ovaries. In comparison, a vasectomy requires only a small skin incision, making the OPU procedure potentially cause more pain.

Importantly, some female marmosets in this study received one dose of maropitant during the postoperative recovery. This was done as a preemptive measure for antiemetic and antinausea effects, based on the preference of the supervising veterinarian. Although there is some evidence suggesting that maropitant may have adjunctive analgesic[34] and antiinflammatory[35] properties, a systemic review and meta-analysis[36] concluded that there is no definitive proof of its effectiveness in reducing inflammation and pain. Notably, no studies have yet investigated these effects in marmosets so far. Also, ketamine[37] and lidocaine[38] are known to have analgesic effects, and in this study one animal received ketamine, and the lidocaine local block was not harmonized across all animals. Therefore, future studies should be stricter about the standardization and uniformity of preemptive drug administration since some of these drugs can have adjunctive antiinflammatory and analgesic effects.

Multimodal analgesic regimens, which target a variety of mechanisms involved in the generation and perception of pain,

are considered to be ideal in marmosets.[6] For example, opioids are most effective at decreasing pain transmitted by C-fiber nociceptors and are best combined with NSAIDs for surgeries that may also cause pain from Aβ fibers. However, within this approach, it remains challenging to isolate and quantify the specific contribution of each individual analgesic to the overall pain relief obtained. Meloxicam is an NSAID that can be used alone or as part of a multimodal regimen to control inflammatory pain and is commonly used in marmosets.[6] However, there are no published pharmacokinetic or efficacy studies regarding meloxicam in marmosets, and the heavy reliance on this medication for surgical pain management requires further research.[6] Therefore, in this study, we opted not to include a treatment group that relied solely on meloxicam. Instead, a multimodal pain regimen was chosen to ensure the best pain control, acknowledging the inherent difficulty in quantifying the individual contributions of buprenorphine and meloxicam in managing pain.

Extended-release buprenorphine formulations have been previously shown to induce a dose-dependent increase in activity in marmosets. Animals exhibited increased locomotor activity following Bup-ER-LAB at 0.15 mg/kg and EXR at 0.15 mg/kg within the home cage compared with saline control.[9] In this study, we also observed an increase in activity and did not classify it as an indicator of pain within the CMPS rubric. This increase in activity has been reported in other species, such as mice,[39] rats,[40,41] and cats.[42] Although there were no statistically significant differences when comparing the drug groups or males compared with females in this study ($P > 0.05$), the increase in activity was more prevalent in males (56%) than females (25%), and could last up to 48 h regardless of sex.

A buprenorphine-associated increase in activity in rodents may result from the activation of mu-opioid receptors, which can lead to dopamine release in brain regions such as the nucleus accumbens and ventral tegmental area.[43] This dopamine release is linked to reward and pleasure,[44,45] potentially causing hyperactivity.[46,47] In addition, activating mu-opioid receptors can suppress GABAergic transmission, influencing dopaminergic activity and contributing to hyperactivity.[48] A similar mechanism may be present in common marmosets; however, additional studies are required to further elucidate the opioid-induced increase in activity in this species.

The objective of our study was to use the ongoing clinical practices by the participating institutions. A previous study in marmosets evaluating extended-release buprenorphine formulations used 0.5 mL tuberculin syringes to administer EXR.[9] However, we did not use a 0.5-mL tuberculin syringe because the largest needle size (permanently attached) for these syringes available at the time of this study was 27-gauge, which is too small to draw up Bup-ER-LAB due to its viscosity. Thus, a 1-mL low dead space syringe, which is currently used by the participating institutions for these viscous formulations, was selected for both drugs. This is a limitation in our study, as it is best practice to choose a syringe size as close as possible to the desired measurement.[49] While the 1-mL syringe size could affect dosing accuracy, the dosing technique used in our study was consistent and performed by 4 well-trained personnel. However, it was not possible to administer the buprenorphine by the same individual since this study was performed at 2 institutions.

Extended-release buprenorphine formulations have been reported to cause injection site reactions in common marmosets,[9] macaques,[50] mice,[51] rats,[52] guinea pigs,[53] and minipigs,[54] typically characterized by localized erythema, swelling, mild ulcerative dermatitis to full-thickness necrosis with concurrent cellulitis, or pyogranulomatous reaction of the skin. Therefore, we elected to include cage-side visual inspections of study animals for potential skin injection site reactions. No medical treatment was needed for any injection site reaction in this study. Although there were no statistically significant differences in injection site reactions when comparing the drug groups or males compared with females in this study ($P > 0.05$), injection site reactions after administration of Bup-ER-LAB (5 out of a total of 7, 2 males and 3 females) was more pronounced than EXR 0.15 mg/kg (2 out of a total of 7, 1 male and 1 female) and EXR 0.1 mg/kg (1 out of a total of 7, 1 female). This observation is consistent with a previous comparison of Bup-ER-LAB compared with EXR in marmosets.[9] In general, skin reactions at the injection site of Bup-ER-LAB are thought to be caused by the drug vehicle. This vehicle is a biodegradable, hydrophobic, water-insoluble polymer that precipitates in body fluids and forms a local depot gel to release buprenorphine slowly. However, the vehicle can trigger an immune response, as the body may perceive it as a foreign body.[55,56] On the other hand, EXR is encapsulated within solid lipid nanoparticles and suspended in a medium-chain fatty acid triglyceride, which undergoes gradual degradation over time through the action of lipase and esterase enzymes.[57] Therefore, a lipid-based vehicle is more similar to biologic membranes and can mimic the natural lipid component of the skin and cause less immune reaction.[58] We did not assess injection site reactions via histopathologic examination; however, in a previous study, the histopathologic evaluation of skin biopsies collected at day 1 and day 10 postdrug administration of Bup-ER-LAB and EXR in marmosets showed that Bup-ER-LAB caused significant injection site reactions compared with saline control sites, while no statistical difference was observed by EXR.[9]

In this study, marmosets undergoing either an OPU or vasectomy that received either Bup-ER-LAB or EXR in a multimodal analgesic regimen experienced minimal to no pain, as determined by a blinded observer via cage-side assessments using our developed CMPS. While future studies are required to validate the CMPS, we hypothesized that a single subcutaneous dose of EXR at 0.1 mg/kg would provide similar analgesia when compared with EXR at 0.15 mg/kg and Bup-ER-LAB at 0.15 mg/kg in marmosets. Based on our findings in this study, we conclude that both long-acting buprenorphine formulations provide effective postoperative analgesia in marmosets at the tested doses. However, Bup-ER-LAB induced a higher incidence of hyperactivity and injection site reactions than did EXR, and EXR 0.1 mg/kg had the least occurrence of these side effects. Also, EXR is an FDA-indexed and pharmaceutical-grade formulation for nonhuman primates, and according to the 8th edition of the *Guide for the Care and Use of Laboratory Animals*, pharmaceutical-grade chemicals and other substances in studies involving experimental animals should be used whenever possible.[59] Therefore, we recommend administering EXR at a dosage of 0.1 to 0.15 mg/kg SC as part of a multimodal analgesic regimen for marmosets undergoing OPU or vasectomy to provide postoperative analgesia with a reduced likelihood of hyperactivity and injection site reactions while also ensuring regulatory compliance.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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References

1. Inoue T, Yurimoto T, Seki F, Sato K, Sasaki E. The common marmoset in biomedical research: experimental disease models and veterinary management. *Exp Anim.* 2023;72(2):140–150.
2. Tardif SD. Marmosets as a translational aging model—introduction. *Am J Primatol.* 2019;81(2):e22912.
3. Kumita W, Sato K, Suzuki Y, et al. Efficient generation of knock-in/knock-out marmoset embryo via CRISPR/Cas9 gene editing. *Sci Rep.* 2019;9(1):12719.
4. Tomioka I, Ishibashi H, Minakawa EN, et al. Transgenic monkey model of the polyglutamine diseases recapitulating progressive neurological symptoms. *eNeuro.* 2017;4(2)ENEURO.0250-16.2017.
5. Tomioka I, Nogami N, Nakatani T, et al. Generation of transgenic marmosets using a tetracyclin-inducible transgene expression system as a neurodegenerative disease model. *Biol Reprod.* 2017;97(5):772–780.
6. Goodroe A, Fitz C, Bakker J. Current topics in marmoset anesthesia and analgesia. *ILAR J.* 2020;61(2–3):218–229.
7. Nunamaker EA, Halliday LC, Moody DE, Fang WB, Lindeblad M, Fortman JD. Pharmacokinetics of 2 formulations of buprenorphine in macaques (Macaca mulatta and Macaca fascicularis). *J Am Assoc Lab Anim Sci JAALAS.* 2013;52(1):48–56.
8. Paterson EA, O'Malley CI, Moody C, Vogel S, Authier S, Turner PV. Development and validation of a cynomolgus macaque grimace scale for acute pain assessment. *Sci Rep.* 2023;13(1):3209.
9. Fabian NJ, Mannion AJ, Jamiel M, et al. Evaluation and comparison of pharmacokinetic profiles and safety of two extended-release buprenorphine formulations in common marmosets (*Callithrix jacchus*). *Sci Rep.* 2023;13(1):11864.
10. Fitz CB, Goodroe AE, Moody DE, Fang WB, Capuano SV. Pharmacokinetics of buprenorphine and sustained-release buprenorphine in common marmosets (*Callithrix jacchus*). *J Am Assoc Lab Anim Sci.* 2021;60(2):188–194.
11. Klein H, Levinson BL, Leary SL, Dobson G. A pharmacokinetic study of extended-release buprenorphine in cynomolgus monkeys (*Macaca fasicularis*). *J Med Primatol.* 2023;52(6):369–373.
12. Escher M, Daali Y, Chabert J, Hopfgartner G, Dayer P, Desmeules J. Pharmacokinetic and pharmacodynamic properties of buprenorphine after a single intravenous administration in healthy volunteers: a randomized, double-blind, placebo-controlled, crossover study. *Clin Ther.* 2007;29(8):1620–1631.
13. Evans HC, Easthope SE. Transdermal buprenorphine. *Drugs.* 2003;63(19):1999–2010; discussion 2011–2012.
14. Sittl R, Griessinger N, Likar R. Analgesic efficacy and tolerability of transdermal buprenorphine in patients with inadequately controlled chronic pain related to cancer and other disorders: a multicenter, randomized, double-blind, placebo-controlled trial. *Clin Ther.* 2003;25(1):150–168.
15. Burns M, Wachtman L. Chapter 10: Physical examination, diagnosis, and common clinical procedures. In: Marini R, Wachtman L, Tardif S, Mansfield K, Fox J, eds. *The Common Marmoset in Captivity and Biomedical Research.* American College of Laboratory Animal Medicine/Academic Press; 2019:145–175.
16. Lussier B, Bakker J, Bouard D, et al. Implementing good practice in aseptic technique for surgery in laboratory animals: Recommendations by the European Academy of Laboratory Animal Surgery (EALAS). IntechOpen. 2024. [Cited 08 January 2025]. http://dx.doi.org/10.5772/intechopen.115098.
17. Bakker J, Roubos S, Remarque EJ, Arndt SS, Kronen PW, Langermans JA. Effects of buprenorphine, butorphanol or tramadol premedication on anaesthetic induction with alfaxalone in common marmosets (*Callithrix jacchus*). *Vet Anaesth Analg.* 2018;45(3):309–319.
18. National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals. Recognition and assessment of pain. In: *Recognition and Alleviation of Pain in Laboratory Animals.* National Academies Press (US); 2009:chapter 3.
19. Langford DJ, Bailey AL, Chanda ML, et al. Coding of facial expressions of pain in the laboratory mouse. *Nat Methods.* 2010;7(6):447–449.
20. Sotocinal SG, Sorge RE, Zaloum A, et al. The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain.* 2011;7:55.
21. Keating SCJ, Thomas AA, Flecknell PA, Leach MC. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. *PLoS One.* 2012;7(9):e44437.
22. Reijgwart ML, Schoemaker NJ, Pascuzzo R, et al. The composition and initial evaluation of a grimace scale in ferrets after surgical implantation of a telemetry probe. *PLoS One.* 2017;12(11):e0187986.
23. Vullo C, Barbieri S, Catone G, et al. Is the piglet grimace scale (PGS) a useful welfare indicator to assess pain after cryptorchidectomy in growing pigs? *Animals.* 2020;10(3):412.
24. Holton L, Reid J, Scott EM, Pawson P, Nolan A. Development of a behaviour-based scale to measure acute pain in dogs. *Vet Rec.* 2001;148(17):525–531.
25. Firth AM, Haldane SL. Development of a scale to evaluate postoperative pain in dogs. *J Am Vet Med Assoc.* 1999;214(5):651–659.
26. Gris VN, Broche N, Kaneko A, et al. Investigating subtle changes in facial expression to assess acute pain in *Japanese macaques*. *Sci Rep.* 2022;12(1):19675.
27. Morozov A, Parr LA, Gothard K, Paz R, Pryluk R. Automatic recognition of macaque facial expressions for detection of affective states. *eNeuro.* 2021;8(6)ENEURO.0117-21.2021.
28. Paterson EA, Turner PV. Challenges with assessing and treating pain in research primates: a focused survey and literature review. *Animals.* 2022;12(17):2304.
29. Miyabe-Nishiwaki T, Gris VN, Muta K, Nishimura R, Mills DS. Primate veterinarians' knowledge and attitudes regarding pain in macaques. *J Med Primatol.* 2021;50(5):259–269.
30. Bartley EJ, Fillingim RB. Sex differences in pain: a brief review of clinical and experimental findings. *Br J Anaesth.* 2013;111(1):52–58.
31. Carbone L. Do 'prey species' hide their pain? Implications for ethical care and use of laboratory animals. *J Appl Anim Ethics Res.* 2020;2(2):216–236.
32. Pieretti S, Di Giannuario A, Di Giovannandrea R, et al. Gender differences in pain and its relief. *Ann Ist Super Sanita.* 2016;52(2):184–189.
33. Murata K, Nozawa K, Matsushita M, et al. Potential sex differences in activation of pain-related brain regions in nonhuman primates with a unilateral spinal nerve ligation. *Neural Regen Res.* 2023;18(11):2466–2473.
34. Marquez M, Boscan P, Weir H, Vogel P, Twedt DC. Comparison of NK-1 receptor antagonist (Maropitant) to morphine as a pre-anaesthetic agent for canine ovariohysterectomy. *PloS One.* 2015;10(10):e0140734.
35. Tsukamoto A, Ohgoda M, Haruki N, Hori M, Inomata T. The anti-inflammatory action of maropitant in a mouse model of acute pancreatitis. *J Vet Med Sci.* 2018;80(3):492–498.
36. Kinobe RT, Miyake Y. Evaluating the anti-inflammatory and analgesic properties of maropitant: a systematic review and meta-analysis. *Vet J Lond Engl 1997.* 2020;259–260:105471.
37. Persson J. Ketamine in pain management. *CNS Neurosci Ther.* 2013;19(6):396–402.
38. Yang X, Wei X, Mu Y, Li Q, Liu J. A review of the mechanism of the central analgesic effect of lidocaine. *Medicine (Baltimore).* 2020;99(17):e19898.
39. Marquez P, Baliram R, Kieffer BL, Lutfy K. The mu opioid receptor is involved in buprenorphine-induced locomotor stimulation and conditioned place preference. *Neuropharmacology.* 2007;52(6):1336–1341.
40. Burke NN, Ferdousi M, Deaver DR, Finn DP, Roche M, Kelly JP. Locomotor and anti-immobility effects of buprenorphine in combination with the opioid receptor modulator samidorphan in rats. *Neuropharmacology.* 2019;146:327–336.
41. Smith MA, Greene-Naples JL, Lyle MA, Iordanou JC, Felder JN. The effects of repeated opioid administration on locomotor activity:

I. Opposing actions of mu and kappa receptors. *J Pharmacol Exp Ther.* 2009;330(2):468–475.
42. Sramek MK, Haas MC, Coleman GD, Atterson PR, Hamlin RL. The safety of high-dose buprenorphine administered subcutaneously in cats. *J Vet Pharmacol Ther.* 2015;38(5):434–442.
43. Soderman AR, Unterwald EM. Cocaine reward and hyperactivity in the rat: sites of mu opioid receptor modulation. *Neuroscience.* 2008;154(4):1506–1516.
44. Baik JH. Dopamine signaling in reward-related behaviors. *Front Neural Circuits.* 2013;7:152.
45. Jeong H, Taylor A, Floeder JR, et al. Mesolimbic dopamine release conveys causal associations. *Science.* 2022;378(6626): eabq6740.
46. Gainetdinov RR, Jones SR, Caron MG. Functional hyperdopaminergia in dopamine transporter knock-out mice. *Biol Psychiatry.* 1999;46(3):303–311.
47. Leo D, Sukhanov I, Zoratto F, et al. Pronounced hyperactivity, cognitive dysfunctions, and BDNF dysregulation in dopamine transporter knock-out rats. *J Neurosci.* 2018;38(8):1959–1972.
48. Wang W, Xie X, Zhuang X, et al. Striatal μ-opioid receptor activation triggers direct-pathway GABAergic plasticity and induces negative affect. *Cell Rep.* 2023;42(2):112089.
49. Jordan MA, Choksi D, Lombard K, Patton LR. Development of guidelines for accurate measurement of small volume parenteral products using syringes. *Hosp Pharm.* 2021;56(3):165–171.
50. Haertel AJ, Schultz MA, Colgin LM, Johnson AL. Predictors of subcutaneous injection site reactions to sustained-release buprenorphine in rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci.* 2021;60(3):329–336.
51. Clark TS, Clark DD, Hoyt RF. Pharmacokinetic comparison of sustained-release and standard buprenorphine in mice. *J Am Assoc Lab Anim Sci.* 2014;53(4):387–391.
52. Nunamaker EA, Goldman JL, Adams CR, Fortman JD. Evaluation of analgesic efficacy of meloxicam and 2 formulations of buprenorphine after laparotomy in female Sprague-Dawley rats. *J Am Assoc Lab Anim Sci.* 2018;57(5):498–507.
53. Smith BJ, Wegenast DJ, Hansen RJ, Hess AM, Kendall LV. Pharmacokinetics and paw withdrawal pressure in female guinea pigs (*Cavia porcellus*) treated with sustained-release buprenorphine and buprenorphine hydrochloride. *J Am Assoc Lab Anim Sci.* 2016;55(6):789–793.
54. Thiede AJ, Garcia KD, Stolarik DF, Ma J, Jenkins GJ, Nunamaker EA. Pharmacokinetics of sustained-release and transdermal buprenorphine in Göttingen minipigs (*Sus scrofa domestica*). *J Am Assoc Lab Anim Sci.* 2014;53(6):692–699.
55. den Dunnen WF, Robinson PH, van Wessel R, Pennings AJ, van Leeuwen MB, Schakenraad JM. Long-term evaluation of degradation and foreign-body reaction of subcutaneously implanted poly(DL-lactide-ε-caprolactone). *J Biomed Mater Res.* 1997;36(3):337–346.
56. Meek MF, Jansen K. Two years after in vivo implantation of poly(DL-lactide-ε-caprolactone) nerve guides: Has the material finally resorbed? *J Biomed Mater Res A.* 2009;89(3):734–738.
57. Navarro K, Jampachaisri K, Huss M, Pacharinsak C. Lipid bound extended release buprenorphine (high and low doses) and sustained release buprenorphine effectively attenuate post-operative hypersensitivity in an incisional pain model in mice (*Mus musculus*). *Animal Model Exp Med.* 2021;4(2):129–137.
58. Mishra DK, Dhote V, Bhatnagar P, Mishra PK. Engineering solid lipid nanoparticles for improved drug delivery: promises and challenges of translational research. *Drug Deliv Transl Res.* 2012;2(4):238–253.
59. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals.* 8th ed. National Academies Press; 2011.