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Important Update:

In order to remain compliant with the most current regulatory guidelines, we have updated the labeling on our SR formulations from Buprenorphine and Meloxicam SR to Buprenorphine and Meloxicam in Polymer. **As of April 1, 2024, SR preparations mentioned in the attached study are now labeled as in Polymer**, with no changes to the formulation of the medication(s).

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ORIGINAL ARTICLE

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The pharmacokinetics and analgesic effects of extended-release buprenorphine administered subcutaneously in healthy dogs

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Buprenorphine is a partial μ agonist opioid used for analgesia in dogs. An extended-release formulation (ER-buprenorphine) has been shown to provide effective analgesia for 72 hr in rats and mice. Six healthy mongrel dogs were enrolled in a randomized, blinded crossover design to describe and compare the pharmacokinetics and pharmacodynamics of ER-buprenorphine administered subcutaneous at 0.2 mg/kg (ER-B) and commercially available buprenorphine for injection intravenously at 0.02 mg/kg (IV-B). After drug administration, serial blood samples were collected to measure plasma buprenorphine concentrations using liquid chromatography/mass spectrometry detection. Heart rate, respiratory rate, body temperature, sedation score, and thermal threshold latency were recorded throughout the study. Median (range) terminal half-life, time to maximum concentration, and maximum plasma concentration of ER-buprenorphine were 12.74 hr (10.43–18.84 hr), 8 hr (4–36 hr), and 5.00 ng/ml (4.29–10.98 ng/ml), respectively. Mild bradycardia, hypothermia, and inappetence were noted in both groups. Thermal threshold latency was significantly prolonged compared to baseline up to 12 hr and up to 72 hr in IV-B and ER-B, respectively. These results showed that ER-buprenorphine administered at a dose of 0.2 mg/kg resulted in prolonged and sustained plasma concentrations and antinociceptive effects up to 72 hr after drug administration.

1 | INTRODUCTION

Buprenorphine is commonly used to provide sedation and analgesia in dogs and other species. The pharmacokinetics and pharmacodynamics of different routes of buprenorphine administration, including intravenous (i.v.) (Morgaz et al., 2013), subcutaneous (s.c.) (Moll, Fresno, Garcia, Prandi, & Andaluz, 2011), intramuscular (i.m.) (Linton, Wilson, Newbound, Freise, & Clark, 2012), transdermal (Moll et al., 2011), and oral transmucosal (Ko et al., 2011), have been previously studied in dogs, with the i.v. and i.m. routes being the most common routes to manage surgical pain in dogs. However, these administration regimens result in dosing every 6 to 8 hr and typically require hospitalization of the patient.

Buprenorphine represents the standard of care for providing analgesia to laboratory rodents (Chum et al., 2014; Cowan, Lewis, & Macfarlane, 1977). Administering injectable analgesia in these species can be challenging, time-consuming, and stressful for the animals, especially when repeated doses are required. Therefore, sustained-release buprenorphine (SR-buprenorphine) formulations have been advocated to provide analgesia of significant duration following a single s.c. injection. Recent studies in mice and rats demonstrated an analgesic effect of 48–72 hr duration following a single s.c. injection of different SR-buprenorphine compounds (Carbone, Lindstrom, Diep, & Carbone, 2012; Chum et al., 2014; Clark, Clark, & Hoyt, 2014; Foley, Liang, & Crichlow, 2011; Healy et al., 2014; Jirkof, Tourvieille, Cinelli, & Arras, 2015; Kendall et al., 2014). Similar SR-buprenorphine compounds have been used in dogs (Nunamaker

et al., 2014; Tomas, Bledsoe, Wall, Davidson, & Lascelles, 2015), cats (Catbagan, Quimby, Mama, Rychel, & Mich, 2011; Enomoto et al., 2017; Johnson et al., 2017), rabbits (DiVincenti, Meirelles, & Westcott, 2016), Göttingen minipigs (Thiede et al., 2014), guinea pigs (Smith, Wegenast, Hansen, Hess, & Kendall, 2016), sheep (Gatson, Pablo, Plummer, & Granone, 2015; Walkowiak & Graham, 2015; Zullian et al., 2016), alpaca (Dooley et al., 2017), elephant seals (Molter et al., 2015), and macaques (Nunamaker et al., 2013). However, the SR-buprenorphine formulations used in these studies were either unknown or compounded products and, in most of these studies, the authors reported skin lesions at the site of injections, ranging from simple s.c. nodules to abscesses, open wounds, and necrotic lesions likely caused by the viscosity of the product or the formulation matrix (Carbone et al., 2012; Catbagan et al., 2011; Clark et al., 2014; DiVincenti et al., 2016; Foley et al., 2011; Molter et al., 2015; Nunamaker et al., 2013, 2014; Thiede et al., 2014).

Because of the limitations and adverse effects associated with the various compounded formulations, a different extended-release solution (ER-buprenorphine) was considered for this study. This formulation has been tested via s.c. injection in mice and rats and provides clinically effective analgesia for 72 hr. In this formulation, buprenorphine is lipid-bound and suspended in medium chain fatty acid triglyceride (MCT) oil. Lipid encapsulation limits the diffusion of buprenorphine, allowing for administration of higher doses of drug within the formulation, decreased toxicity, and prolonged activity of opioid therapy (Bethune, Bernards, Bui-Nguyen, Shen, & Ho, 2001; Mishra, Dhote, Bhatnagar, & Mishra, 2012).

Currently, there are no buprenorphine formulations approved for use in the United States for dogs, and limited pharmacokinetic data of SR-buprenorphine in this species are available (Nunamaker et al., 2014). A safe, long-acting formulation of buprenorphine, which provides effective analgesia for surgical procedures, would be ideal for dogs that do not require prolonged hospitalization and can be discharged immediately after or within 24 hr of the procedure. The objective of this study was to determine the pharmacokinetics and analgesic effects of the ER-buprenorphine in dogs and compare this formulation to a standard dose of intravenous buprenorphine. A second objective was to evaluate systemic and local side effects (at the injection site) of the ER-buprenorphine. The hypotheses were that plasma concentrations greater than 1.0 ng/ml would be detected up to 72 hr after the s.c. administration of ER-buprenorphine and would correlate with an increase in the thermal threshold withdrawal latency, without any serious systemic or local side effects.

2 | MATERIALS AND METHODS

2.1 | Animals

Six healthy mongrel dogs including three castrated males and three intact females (1.5–5 years of age; mean ± *SD* body weight of 28.7 ± 6.7 Kg) were enrolled in this study. All animals were assessed as healthy on the basis of results of physical examination, complete blood counts, and serum chemistry panels. Dogs were housed in the North Carolina State University Laboratory Animal Resources facility, where a maintenance diet was provided twice daily and water was provided ad libitum. Animals were allowed to acclimate for a minimum of 7 days before the beginning of the data collection. The study was reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University (protocol number 14–171).

2.2 | Study design/treatments

A prospective, randomized, blinded, within-subjects crossover experimental design was used for this study. Dogs were assigned by a random number generator (GraphPad Prism 6, GraphPad Software

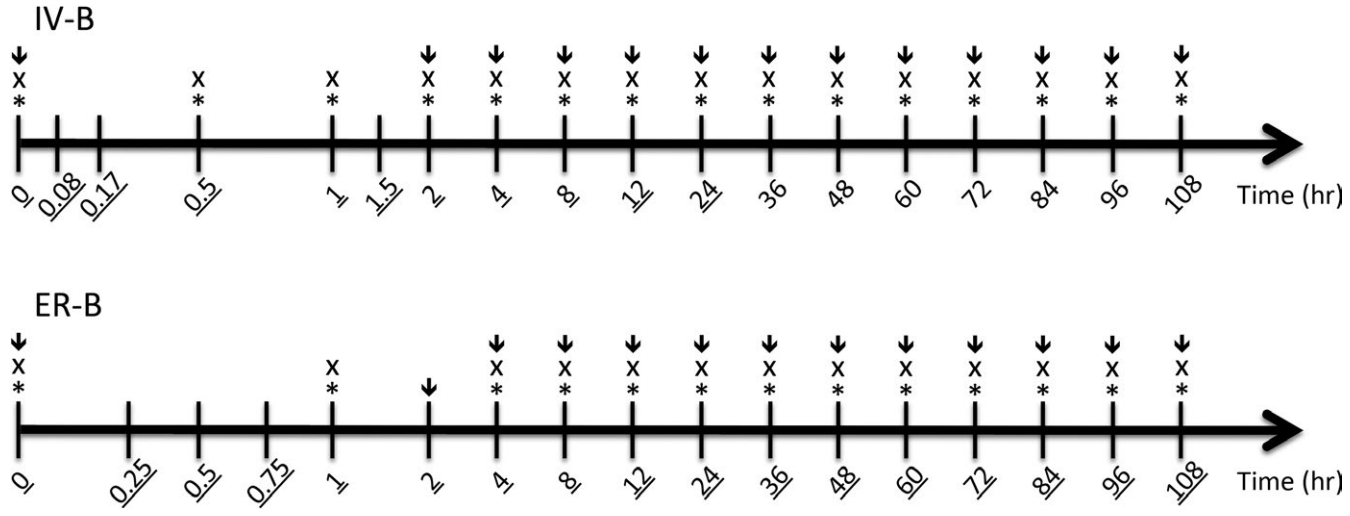

FIGURE 1 Timeline on study activities. The underlined time points represent blood collection for buprenorphine plasma concentrations. Physiological variables (respiratory rate, heart rate, and rectal temperature), sedation score, and thermal threshold latency data were collected at the time points marked with ↓, X, and *, respectively

Inc., La Jolla, CA, USA) to receive either i.v. buprenorphine HCl (IV-B) (Hospira Inc., Lake Forest, IL, USA) at a dose of 0.02 mg/kg, or s.c. extended-release buprenorphine (ER-B) (Animalgesic Labs, Millersville, MD, USA) at a dose of 0.2 mg/kg. The dose of the ER-buprenorphine was determined after consultation with the pharmaceutical company providing the ER-B (Animalgesic Labs), extrapolated from pharmacokinetic data obtained in rats, and tested in a pilot study using two dogs that showed efficacy based on the thermal withdrawal model used, and detectable plasma concentrations up to 72 hr after administration. After a washout period of at least 14 days, the study was repeated and dogs were administered the other treatment. The timeline of the study is described in Figure 1.

2.3 | Instrumentation

Approximately 48 hr prior to the study, the dogs were fasted for 12 hr in preparation to the jugular catheter placement. Dogs were sedated with 10 μg/kg dexmedetomidine i.v. (Dexdomitor; Zoetis, Kalamazoo, MI, USA) and 1 ml of 2% lidocaine (Lidocaine HCl; Hospira, IL, USA) was administered s.c. for local anesthesia over the jugular vein. A jugular catheter (JorVet; Jorgensen Laboratories, Inc, Loveland, CO, USA) was placed aseptically using the Seldinger technique. The catheters were secured in place with suture, and a light bandage was applied to avoid displacement. After placement and during the study, the jugular catheters were flushed with sterile 0.9% saline and locked with heparinized saline to maintain patency. At the end of the catheterization procedure, atipamezole 0.1 mg/kg (Antisedan; Zoetis, Kalamazoo, MI, USA) was administered intramuscularly to reverse the dexmedetomidine.

2.4 | Drug administration and blood collection

Dogs were fasted for 12 hr prior to the study. Before drug administration, a minimum of 3 ml of blood was collected from the jugular catheter of each dog and the plasma was used as negative controls for the buprenorphine assay. A 20 gauge i.v. catheter (Surflo 1.25 inch; Terumo Medical Corporation, Elkton, MD, USA) was placed in the right cephalic vein and secured with tape and light bandage, for administration of buprenorphine in dogs in the IV-B group. This catheter was removed immediately after drug administration, and a pressure bandage was applied. All animals, regardless of treatment group, received this pressure bandage to ensure that the person scoring sedation and thermal threshold remained blinded to the treatment. The ER-buprenorphine was administered s.c. in the interscapular region using a 20 gauge needle. Drug doses were collected in syringes immediately prior to administration.

Blood samples for dogs in the IV-B group were collected at 0 (pretreatment), 0.08, 0.17, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, and 24 hr postdrug administration. Blood samples of dogs in the ER-B group were collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr after the administration of the drug. These time points were determined based on the preliminary data collected from the two pilot dogs, not included in this study, after analysis of the plasma concentration–time profile. Upon collection, samples were transferred into tubes containing lithium heparin as anticoagulant (BD Vacutainer; Franklin Lakes, NJ, USA) and the tubes were immediately placed in ice. Samples were centrifuged at 4500 × *g* for 10 min at 20°C within 60 min of collection. The plasma from centrifuged samples was separated and stored at −80°C until analysis within 1 month of collection. After the final blood sample collection, the jugular catheters were removed, and a light pressure wrap was placed around the neck for a minimum of 30 min.

During the study and up to 4 weeks after data collection, the animals were monitored twice daily for signs of reaction to the s.c. injection of ER-buprenorphine, such as redness, swelling, pustules, ulcerations, and pain upon palpation.

2.5 | Physiologic variables

Before the administration of i.v. or s.c. extended-release buprenorphine, baseline (time 0) respiratory rate (RR), heart rate (HR), and rectal temperature (T) were recorded. These variables were also measured and recorded 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr after the administration of the drug. If these measurements, sedation score (SS), thermal threshold latency (TTL), and collection of blood for buprenorphine plasma concentrations occurred at the same time point, the physiologic variables were always measured first, immediately followed by blood collection and 10 min later by SS and TTL. Normal limits for the monitored variables were defined as RR = 15–30 breaths/min, HR = 70–120 beats/min, and T = 37.5–39.2° C (Haskins et al., 2005; Plumb, 2015). Other clinical signs, such as demeanor/attitude, appetite, emesis, defecation, urination, and salivation, were monitored and recorded throughout the entire study period. The dog's regular food (Hill's Science Diet Adult Large Breed-Dry) was offered 2 hr after the drug administration, and the consumption was noted. If the animal did not show interest in the dry food within 24 hr, an alternative diet (Hill's Prescription Diet a/d Canine/Feline-Canned) was offered, followed by boiled chicken if the dog did not eat anything 36 hr after the injection of buprenorphine. The presence of feces and urine in the cage was monitored throughout the study, but the amount of feces and urine output was not measured or recorded at every time point.

2.6 | Sedation score

During the acclimation period, the investigators interacted with the dogs at least twice daily, to learn the demeanor of each subject and allow the dogs to become accustomed to the evaluators. Before the beginning of each trial, a baseline SS (time 0) was assigned by use of a modified standardized sedation scoring system (Smith, Yu, Bjorling, & Waller, 2001), previously used in another study (Hofmeister, Chandler, & Read, 2010) (Appendix), where 0 was normal behavior, negative numbers represented agitation/dysphoria, and positive numbers sedation. A SS was also obtained at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr and 0, 1, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr after drug administration for IV-B and

ER-B, respectively. Briefly, each dog was first observed undisturbed by a blinded investigator (AT), who scored vocalization, posture, and appearance. After this phase, the animal was approached, spoken to, touched, and gently restrained by the same investigator and interactive behavior and restraint were recorded. Lastly, the response to noise was evaluated by observing the reaction of the animal to a handclap near the head. The scoring procedure was performed in the same order and required approximately the same amount of time for each dog.

2.7 | Thermal threshold

The Canine Thermal Escape System (CTES) was used to test the TTL in this study according to previously described methods (Wegner et al., 2008; Williams et al., 2014). The apparatus was purchased from the laboratory that originally described the system (Wegner et al., 2008) and was used in a previous study by one of the authors (DL) (Williams et al., 2014).

Dogs were acclimated to the CTES twice daily for 7 days prior to initiation of the study. The animals were required to maintain a "square" position during the training period and the testing procedure as described elsewhere (Williams et al., 2014). The left and right hind limb feet were alternatively placed on the glass plate and left for approximately 60 s. A blinded operator stood on the left of the device and gently touched the dog's inguinal area, without supporting any weight, to encourage the animal to stand still. A second person, the blinded evaluator (AT), sat on the right of the device, where the controller for the halogen bulb and the digital timer were located, to make sure that the animals would be accustomed to his presence during the testing procedure. The temperature of the glass was tested before the experiment and it measured 54°C and 59°C at 20 and 30 s, respectively, which was similar to the temperature reported in previous studies (Wegner et al., 2008; Williams et al., 2014). Before the administration of buprenorphine, a baseline TTL (time 0) was recorded for each trial. Thermal threshold latency measurements were also obtained at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr and 0, 1, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hr after drug administration for IV-B and ER-B, respectively. The dog was positioned on the CTES as described above, and the light source was positioned under the center of digital pad III. This location was chosen because previous data showed that this pad had more consistent contact with the glass plate (Williams et al., 2014). The thermal stimulus was then initiated and automatically terminated after 40 s or when the dog lifted the foot from the glass plate, whichever occurred first. The time between the initiation and termination of the stimulus was recorded by the blinded evaluator as TTL. This procedure was repeated 3 times per subject for each time point alternating the hind limbs, and the first foot tested was randomly selected using a coin toss. Two minutes were allowed between measurements and, during this time, the dog was allowed to move freely, sit, or stand still on the device. If the variation among any of these TTL values was greater than 20%, a fourth measurement was obtained. The average of these values recorded for each dog was used as TTL for that specific time point.

2.8 | Buprenorphine analysis

Canine plasma samples were analyzed by ultra-high-pressure liquid chromatography (UPLC) followed by detection with tandem mass spectrometry using a method developed in the authors' laboratory and modified for canine plasma samples (Gulledge, Messenger, Cornell, Lindell, & Schmiedt, 2017; Messenger, Davis, LaFevers, Barlow, & Posner, 2011). Calibration curves and quality controls were prepared by fortifying blank canine plasma with stock solutions of buprenorphine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 100% methanol. Plasma samples, standards, and quality controls were then prepared by adding 500 µl to 500 µl of 2% ammonium hydroxide in water in a glass tube, and vortexing for 15 s. The sample mixture was then added to 1 ml supported liquid extraction cartridges (Isolute SLE+, Biotage, Uppsala, Sweden), and a light vacuum was applied to initiate absorption. Two aliquots of 2.5 ml of methyl tert-butyl ether (Fisher Scientific, Pittsburgh, PA) were added to the cartridges, allowed to sit for 5 min, and then slowly eluted through under light vacuum. The resulting eluate was then placed in an evaporator and dried under a 20 psi stream of air for 20 min at 45°C. Samples were reconstituted in 150 µl of 50:50 acetonitrile: water (v/v) containing 0.1% formic acid. All samples were filtered into injection vials using 0.2 µm nylon syringe filters (4-mm Nalgene filters). Volumes of 8 µl for samples and standards were injected on an Acquity I-Class UPLC with an Acquity Xevo TQD mass spectrometer (MS/MS) (Waters Corporation, Milford, MA, USA) using a flow rate of 0.3 ml/min. A gradient was used, and the initial mobile phase was 0.1% formic acid in water: 0.1% formic acid in acetonitrile (85:15 v/v) for the first 2.5 min. The mobile phase then switched to (10:90 v/v) from 2.5 to 4 mins. The last 1 min of the run, the mobile phase was (85:15 v/v). The Xevo TQD was run in ESI+ mode. The quantification MRM transition was 468.39 > 100.89, and the qualifier MRM transition was 468.39 > 83.73. Column temperature was maintained at 40°C, and sample temperature was maintained at 10°C. Separation was achieved using an Acquity UPLC HSS T3 column (1.8 µm, 2.1 × 100 mm) and VanGuard guard column (Waters Corporation). The retention time observed for buprenorphine was 1.48 min. Standard curves were linear over a concentration range of 0.1–25 ng/ml with an $R^2 \geq .99$ daily. The lower limit of quantification was 0.1 ng/ml. Accuracy (% nominal concentration) and precision (% relative standard deviation) were determined from quality control samples fortified with buprenorphine at concentrations of 0.2, 2, and 20 ng/ml ($n = 6$ for each concentration). Accuracy ranged from 100% to 109%, and precision ranged from 4% to 13%.

2.9 | Pharmacokinetic analysis

Concentration versus time data for each dog were plotted and visually inspected. Pharmacokinetic analysis was performed using commercially available software (Phoenix WinNonLin, ver 6.3,

Certara, St. Louis, MO, USA). Noncompartmental analysis was performed for both the i.v. and s.c. routes of administration. The area under the curve from time zero to infinity ($AUC_{0-\infty}$) and the area under the first moment curve to infinity ($AUMC_{0-\infty}$) were calculated using the linear up log down trapezoidal method. Standard noncompartmental equations were used to calculate other parameter estimates, including clearance, volume of distribution at steady state, mean residence time, first-order rate constant (λ_z), and terminal half-life (Gabrielsson & Weiner, 2006; Gibaldi & Perrier, 1982). Values for C_{MAX} and T_{MAX} were taken directly from the data.

2.10 | Statistical analysis

Normality of the data was assessed based on examination of a histogram, normal plot of the residuals, and with the Shapiro–Wilk test. All variables met assumptions for parametric analyses except for RR and T data, which were log-transformed for statistical analysis. A

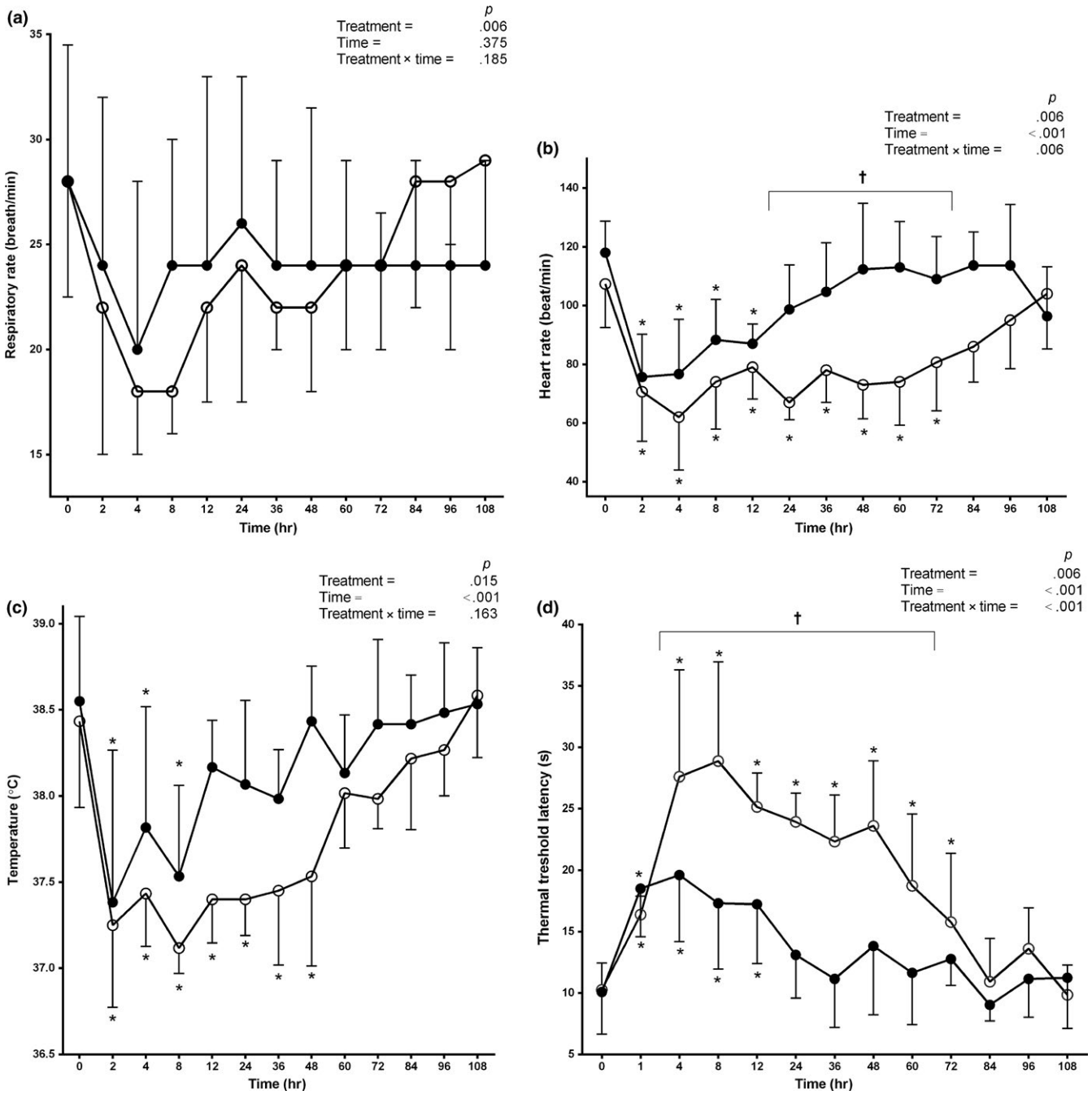

FIGURE 2 Mean ± *SD* respiratory rate (panel a), heart rate (panel b), body temperature (panel c), and thermal threshold latency (panel d) observed in six healthy mongrel dogs (three castrated males and three intact females) after administration of a single dose of i.v. buprenorphine at 0.02 mg/kg (●) and subcutaneous extended-release buprenorphine at 0.2 mg/kg (○). *Indicates a significant difference from baseline within the group. †Indicates a significant difference between the two groups

two-way repeated-measures ANOVA with Sidak's *post hoc* analysis was used to compare RR, HR, T, TTL, and SS. Time to resume normal food consumption between the two groups was compared using the Wilcoxon signed-rank test for paired samples. The correlation coefficients between plasma concentrations of buprenorphine and physiologic variables (HR, RR, and T), SS, and TTL were calculated using the method for repeated observations previously described (Bland & Altman, 1995). All analyses were carried out with two commercially available statistical software programs (Prism version 6.0; GraphPad Software, Inc., CA, USA and JMP Pro 12.1; SAS Institute Inc., NC, USA). Parametric values were expressed as mean ± *SD*, and nonparametric values were expressed as median (IQR). Significance was set at $\alpha < .05$.

3 | RESULTS

Mean ± *SD* age and body weight were 3.2 ± 1.2 year and 28.7 ± 6.7 kg, respectively. One dog developed a skin reaction around and ventral to the jugular catheter insertion site at the end of the study, which resolved with medical management including oral carprofen (Rimadyl; Zoetis, Kalamazoo, MI, USA 2.2 mg/kg BID), and local application of antibiotic ointment (Triple Antibiotic Ointment; Actavis, Parsippany, New Jersey, USA).

No complications were recorded during administration of both drug formulations, and no pain upon injection of ER-buprenorphine was noted. The results for the physiologic variables are reported in Figure 2.

Three dogs in IV-B group were reluctant to stand and walk 30 min after drug administration and one of these subjects maintained this behavior for up to 2 hr. Another dog in this group presented signs of agitation, such as constant walking and whining, and appeared nervous between 3 and 4 hr after the injection. Four dogs in the IV-B group ate their dry diet when first presented 2 hr after drug administration; the other two subjects showed interest at 8 and 12 hr, respectively. Four dogs in ER-B presented unwillingness to stand and walk at 1 hr ($n = 1$), 4 hr ($n = 1$), and up to 12 ($n = 1$) and 36 hr ($n = 1$) after receiving ER-buprenorphine. None of the dogs showed interest in food at 2 hr, and only one subject resumed eating at 4 hr and another three dogs at 8 hr after drug administration. The remaining two dogs in this group started eating 24 hr and 60 hr postinjection. The median time required to resume normal food consumption was 2 hr (range, 2–12 hr) and 16 hr (range, 4–72 hr) in IV-B group and ER-B group, respectively. Dogs in ER-B group showed a significantly prolonged decrease in time to food consumption compared to the IV-B group ($p = .03$). The presence of urine and fecal material was noted in the cage for all dogs of each group within 12 hr of i.v. and s.c. buprenorphine administration.

Mean SSs were 1.1 ± 0.8 and 1.0 ± 1.0 for IV-B and ER-B, respectively. There was a significant effect of time ($p = .002$) but no effect of drug ($p = .497$) on the SSs. Overall, dogs appeared more sedated only 4 hr after administration of buprenorphine compared to baseline regardless of the treatment group ($p = .016$).

Mean TTL at baseline was 10.1 ± 3.9 s and 10.2 ± 2.2 s for IV-B and ER-B, respectively, and no difference was found between groups ($p > .999$; Figure 2d). Compared to these baseline values, TTL was significantly longer for up to 12 hr in the IV-B group and 72 hr in the ER-B group ($p < .001$).

The pharmacokinetics results for IV-B group and ER-B group are reported in Table 1, and the plasma concentrations *vs* time curves of both groups are displayed in Figure 3. The correlation coefficients between plasma concentration of buprenorphine and RR, HR, and T were −0.41 ($p < .001$), −0.41 ($p < .001$), and −0.56 ($p < .001$), respectively. When TTL and SS were considered, the correlation coefficients were 0.68 ($p < .001$) and 0.38 ($p < .001$), respectively.

No signs of reaction (pain upon injection, swelling, injection site reaction) to the s.c. injection of ER-buprenorphine were noted during and after the study and all subjects completed the study without long-term complications related to the drug.

TABLE 1 Pharmacokinetics values (median and range) determined from six healthy mongrel dogs (three castrated males and three intact females) after administration of a single dose of intravenous buprenorphine (IV-B) at 0.02 mg/kg and subcutaneous extended-release buprenorphine (ER-B) at 0.2 mg/kg

		IV-B		ER-B	
Parameter	**Units**	**Median**	**Range**	**Median**	**Range**
$AUC_{0-\infty}$	hr*ng/ml	14.69	10.23–22.88	223.97	194.29–450.47
$AUC_{\%\ ext}$	%	9.46	2.95–14.07	2.48	1.16–5.77
λ_z	1/h	0.17	0.15–0.39	0.05	0.03–0.07
$T\ ½_{\lambda z}$	H	3.97	1.77–4.59	12.74	10.43–18.84
MRT	H	3.36	1.88–4.75	37.19	25.84–47.02
Cl	ml/min/kg	23.32	14.57–32.59	n/a	n/a
Vss	ml/kg	4680.1	2549.4–7637.6	n/a	n/a
T_{max}	H	n/a	n/a	8	4–36
C_{max}	ng/ml	n/a	n/a	5.00	4.29–10.98

$AUC_{\%\ ext}$, Percent of the area under the curve extrapolated to infinity; $AUC_{0-\infty}$, Area under the concentration versus time curve from zero to infinity; Cl, Clearance; C_{MAX}, Maximum plasma concentration; MRT, Mean residence time; $T\ ½_{\lambda z}$, Terminal half-life; T_{MAX}, Time to maximum concentration; V_{ss}, Volume of distribution at steady state; λ_z, Elimination rate constant.

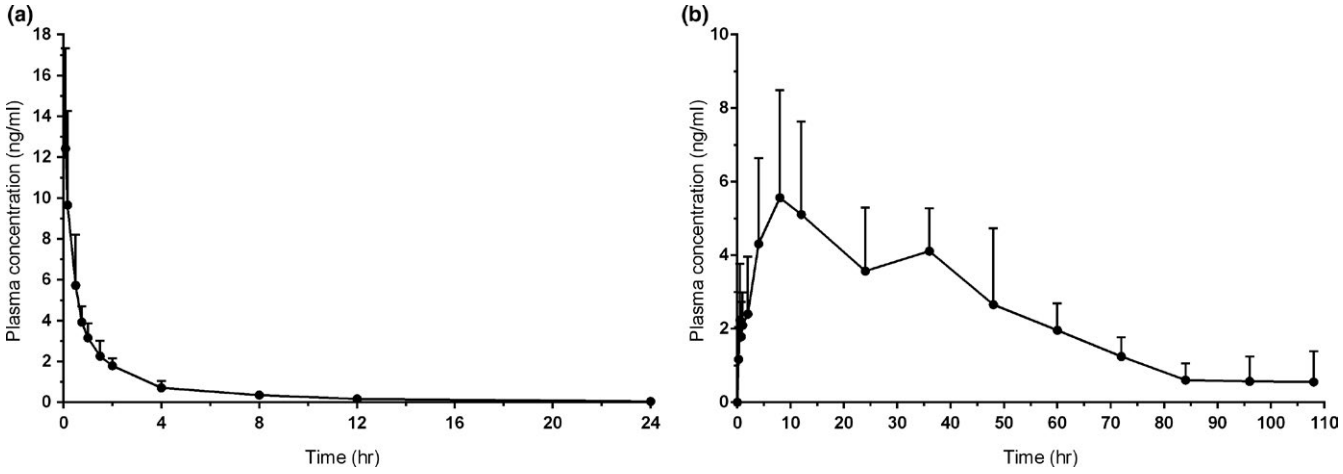

FIGURE 3 Mean ± *SD* plasma concentration versus time measured in six healthy mongrel dogs (three castrated males and three intact females) after administration of a single dose of intravenous buprenorphine at 0.02 mg/kg (panel a) and subcutaneous extended-release buprenorphine at 0.2 mg/kg (panel b)

4 | DISCUSSION

Different formulations of compounded SR-buprenorphine have been used in several laboratory, companion, and farm animal species, with variable results and effects. In many of these studies, the authors reported different types of skin lesions most likely due to reaction to the vehicle (Carbone et al., 2012; Catbagan et al., 2011; Clark et al., 2014; DiVincenti et al., 2016; Foley et al., 2011; Molter et al., 2015; Nunamaker et al., 2013, 2014; Thiede et al., 2014). Additionally, reports of inconsistent systemic absorption following use of this formulation have been described (Dooley et al., 2017). The buprenorphine used in these papers is formulated in a copolymer of lactide and caprolactone dissolved in a biocompatible solvent. Upon injection, the polymer precipitates forming an implant matrix from which the drug is slowly released. In contrast, in the present study, we utilized a lipid-encapsulated buprenorphine formulation which has been studied in laboratory rodents. This delivery system for buprenorphine has been previously described in rats (Misra & Pontani, 1978), and no side effects were noted after an observation period of 4 months (Pontani & Misra, 1983). The same formulation used in the current study was administered to 344 rats and the authors did not observe any macroscopic or microscopic skin lesions at the injection site (Cowan, Sarabia-Estrada, Wilkerson, McKnight, & Guarnieri, 2016). Another study tested this vehicle in 120 mice and found no evidence of clinical and histological lesions after an observation period of up to 12 days (DeTolla, Sanchez, Khan, Tyler, & Guarnieri, 2014). The formulation used was developed/compounded by the authors' of that study, and the vehicle successfully delivered the drugs with no signs of adverse reaction noted at the site of injection (Johnson et al., 2017). In the current study, none of the dogs exhibited any signs of skin reaction around the inoculation area and all dogs maintained detectable plasma concentrations at least up to 72 hr after administration, suggesting this formulation provides reliable systemic absorption of buprenorphine with sustained release of the drug over time.

The pharmacokinetics of a lipid-encapsulated extended-release formulation of buprenorphine have not been previously described in dogs. The C_{MAX} and T_{MAX} of this formulation, when injected s.c., are similar to values reported in Beagle dogs following administration of a different sustained release formulation at the same dose (0.2 mg/kg) (Nunamaker et al., 2014). However, the AUC and estimated elimination half-lives are different. In the present study, the AUC was larger than that reported by Nunamaker and others (224 hr*ng/ml versus 189 hr*ng/ml), although the elimination half-life was shorter (approximately 13 hr in our study, versus 64 hr in the study reported by Nunamaker). One difference in the AUC reported between the two studies is due to differences in study design and the reporting of the AUC; Nunamaker reported the $AUC_{0\text{-}168\ h}$, whereas we reported the $AUC_{0\text{-}\infty}$ and only sampled to 108 hr. However, other differences are in the overall concentrations of buprenorphine which explain the differences; in our study, higher sustained plasma concentrations of buprenorphine (> 2 ng/ml) were noted for 48 hr after injection, whereas in the study reported by Nunamaker, levels were much lower, around 1 ng/ml during this time. In addition, the buprenorphine concentrations declined much more slowly in the study by Nunamaker and were still above 0.5 ng/ml up to 136 hr postinjection, whereas in our study, mean levels reached 0.5 ng/L at the 96–108 hr time points. The difference in the pharmacokinetics between these two formulations suggests that formulation factors could play a role systemic drug absorption, although pharmacokinetic studies involving a larger number of dogs are needed.

Therapeutic plasma concentrations of buprenorphine are variable among different species and even within individuals of the same species (Evans & Easthope, 2003; Ko et al., 2011; Nunamaker et al., 2013, 2014). In humans, buprenorphine plasma concentration as low as 0.1 ng/ml have been associated with analgesia (Sittl, Griessinger, & Likar, 2003). The correlation between plasma concentration and clinical efficacy of buprenorphine has not been clearly identified in dogs or other companion animal species. In one study, the authors noted that plasma concentrations of 0.6 ng/ml were detected in

seven of nine dogs requiring rescue analgesia after ovariohysterectomy, suggesting that plasma concentrations greater than 0.6 ng/ml are needed for therapeutic efficacy following soft tissue surgery (Ko et al., 2011). This plasma level was used as reference in a recent study evaluating the effects of a sustained release formulation of buprenorphine combined with meloxicam on postoperative analgesia in dogs undergoing ovariohysterectomy, which found that some dogs required rescue analgesia at plasma levels of 2 ng/ml, while other dogs were deemed comfortable although buprenorphine plasma concentrations were less than 0.1 ng/ml (Nunamaker et al., 2014). In another study, it was reported that, despite similar plasma concentrations, i.v. administration of buprenorphine was associated with a greater increase in TTL when compared to a transdermal formulation. The authors concluded that the larger diffusion gradient between plasma and central nervous system in the i.v. group contributed to this difference (Pieper, Schuster, Levionnois, Matis, & Bergadano, 2011). In the current study, 5/6 dogs in ER-B reached plasma concentrations above 1 ng/ml between 15 and 30 min after drug administration. Plasma concentrations dropped below 0.6 ng/ml in all subjects between 4–8 hr and 60–84 hr in IV-B and ER-B, respectively, while TTL was significantly longer compared to baseline values up to 12 hr and 72 hr in IV-B and ER-B, respectively. All plasma concentrations were between 0.14–0.3 ng/ml at 12 hr and 0.9–1.8 ng/ml at 72 hr in IV-B and ER-B, respectively. As others have suggested, it is possible that when buprenorphine is administered i.v. the larger diffusion gradient allows for faster movement and greater amount of the drug into the central nervous system, which may result in a longer duration of action. This may correlate with lower plasma concentrations required to see a clinical effect. Based on the results of the present study, when an extended-release formulation is injected s.c., plasma concentration of at least 1 ng/ml might be required to see similar effects.

Both RR and HR were poorly correlated with plasma concentrations of buprenorphine. During data collection of the physiologic variables, some dogs were more excited than others, even if the level of sedation subjectively appeared similar when the animals were left undisturbed. It is possible that regardless the level of plasma concentration, if the animals were visually stimulated, they moved around and the RR and HR increased resulting in poor correlation between the two variables. It is also possible that plasma concentrations of buprenorphine do not correlate to the clinical effect of the drug on physiological variables.

The level of sedation was evaluated using a scale developed elsewhere (Hofmeister et al., 2010; Smith et al., 2001). This scale seems to be particularly useful to evaluate the effect of opioids in dogs as it allows the investigator to capture not only sedation (high positive score) but also excitement and dysphoria (negative score). Although there was a higher score in sedation in ER-B compared to IV-B, the difference between the two groups was not significant. It is likely that the presence of the evaluator interfered with the level of sedation. To minimize this bias, during the acclimation period, the evaluator interacted with the dogs several times a day and collected physiologic variables, SS, and TTL to train the animals for the study. Ten minutes were allowed between data collection of physical variables and SS to calm the animal in case excitement occurred during this phase. It was decided to collect data from the physical variables before the SS due to the interactive nature of this scale. As HR, RR, and T are more objective data compared to the SS, the authors wanted to prioritize them by eliminating any stimulation necessary to assess the level of sedation. Despite these efforts, it was evident that some dogs that subjectively appeared sedated from a distance became aroused and excited during visual stimulation and interaction with the evaluator. This could also explain why there was only low positive correlation between SS and buprenorphine plasma concentration.

A canine thermal escape model, the CTES, has been validated in dogs to test TTL after receiving analgesic and sedative drugs, such as hydromorphone, morphine, fentanyl, buprenorphine, butorphanol, dexmedetomidine, and acepromazine (Wegner et al., 2008) and was deemed to be feasible and repeatable for assessing pain in healthy, client-owned dogs and in dogs with osteoarthritis without previous training (Williams et al., 2014). The same system was used in this study to quantify the duration of the effects of buprenorphine on TTL. In previous studies using the CTES, the thermal stimulus was applied 2 to 5 times to each paw (Wegner et al., 2008; Williams et al., 2014). Due to the high number and frequency of data collection points, three TTL measurements were taken each time. To decrease the chance of thermal injury, the pelvic limbs were alternated. This decision was supported by the study by Wegner et al. (2008), where they found no difference between the two limbs. These authors also showed that the CTES was reliable in assessing analgesia induced by opioids, including buprenorphine, and was able to differentiate between opioid-induced analgesia and sedation produced by acepromazine. These results are in agreement with the current study. The TTL data were also strongly correlated to the plasma concentrations of the drug, which confirms that the CTES is a valuable method to assess opioid-induced analgesia in dogs.

There are some limitations to this study. Sedation was evaluated after collection of physiological variables and blood. To minimize any interference, the dogs were trained to this sequence for 7 days before the experiment and 10 min were allowed before SS was assessed. Despite these efforts, the SS might have not reflected the real sedation level of these dogs. Videotapes could have been used for this purpose, but this would have negated direct interaction with the animals. In addition, only six healthy dogs were enrolled in this study. Although the results reported here are promising, it is not possible to determine clinical conclusions based on these data. A clinical study with a larger number of subjects, including animals undergoing surgical procedures, should be conducted to determine the effects of ER-buprenorphine in naturally occurring pain in dogs.

5 | CONCLUSIONS

The results of this study showed that the novel formulation of ER-buprenorphine used in this experiment can be administered to healthy dogs, and the antinociceptive effects and hypothesized therapeutic plasma concentrations of 1 ng/ml were observed for at least 72 hr after

administration of 0.2 mg/kg s.c. Antinociceptive effects, based on TTL, were strongly correlated to plasma concentrations, but physiologic variables and SSs were not correlated with plasma concentrations. Side effects included bradycardia, hypothermia, and inappetence. Based on these results, further studies investigating the analgesic effects of this novel formulation in clinical cases are warranted.

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CONFLICT OF INTEREST

The authors declare no conflict of interests related to this study.

AUTHOR CONTRIBUTIONS

MB conceived and designed study, performed in vivo studies, performed statistical analysis, drafted manuscript, and approved final manuscript. SO and ACT performed in vivo studies and buprenorphine analysis, drafted manuscript, and approved final manuscript. JQ and BDXL participated in study design and data analysis, drafted manuscript, and approved final manuscript. KM conceived and designed study, performed in vivo studies, performed PK analysis, drafted manuscript, and approved final manuscript.

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APPENDIX

Observation	Score	Description
Vocalization	0	Quiet
	−1	Whining softly but quiets with soothing touch
	−2	Whining continuously
	−3	Barking continuously
Posture	3	Lateral recumbency
	2	Sternal recumbency
	1	Sitting or ataxic while standing
	0	Standing
	−1	Moving continuously
Appearance	3	Eyes sunken, glazed, or unfocused; ventromedial rotation
	2	Eyes glazed but follow movement
	1	Protrusion of nictitating membrane; normal visual responses
	0	Normal appearance
	−1	Pupils dilated; abnormal facial expression
Interactive behavior	3	Recumbent; no response to voice or touch
	2	Recumbent; lifts head in response to voice or touch
	1	Recumbent but stands in response to voice or touch
	0	Standing or sitting up; normal response to voice or touch
	−1	Moves away from voice or touch; appears anxious
	−2	Growls when approached or touched
	−3	Bites when approached
Restraint	2	Lies on floor with minimal restraint
	1	Lies on floor with light restraint of head or neck
	0	Sits up on floor; attempts to jump despite restraint
	−1	Struggles continuously against restraint
	−2	Cannot be restrained for > 20 seconds
Response to noise	3	No response to a handclap near the head
	2	Minimal response to a handclap near the head
	1	Slow or moderate response to a handclap near the head
	0	Brisk response to a handclap near the head; raises head with eyes open