COMPARISON OF KETAMINE-XYLAZINE, BUTORPHANOL-AZAPERONE-MEDETOMIDINE, AND NALBUPHINE-MEDETOMIDINE-AZAPERONE FOR RACCOON (PROCYON LOTOR) IMMOBILIZATION

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Comparison of Ketamine-Xylazine, Butorphanol-Azaperone-Medetomidine, and Nalbuphine-Medetomidine-Azaperone for Raccoon (*Procyon lotor*) Immobilization

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ABSTRACT: Raccoons (*Procyon lotor*) are frequently handled using chemical immobilization in North America for management and research. In a controlled environment, we compared three drug combinations: ketamine-xylazine (KX), butorphanol-azaperone-medetomidine (BAM), and nalbuphine-medetomidine-azaperone (NalMed-A) for raccoon immobilization. In crossover comparisons, raccoons received a mean of the following: 8.66 mg/kg ketamine and 1.74 mg/kg xylazine (0.104 mL/kg KX); 0.464 mg/kg butorphanol, 0.155 mg/kg azaperone, and 0.185 mg/kg medetomidine (0.017 mL/kg BAM); and 0.800 mg/kg nalbuphine, 0.200 mg/kg azaperone, and 0.200 mg/kg medetomidine (0.020 mL/kg NalMed-A). Induction time was shortest with KX (mean±SE, 10.0±0.7 min) and longest with NalMed-A (13.0±1.3 min). A sampling procedure was completed on 89% (16/18), 72% (13/18), and 89% (16/18) of the raccoons administered KX, BAM, and NalMed-A, respectively. Reasons for incomplete sampling included inadequate immobilization (one KX and one NalMed-A), responsive behaviors (one each with KX, BAM, NalMed-A), or animal safety (four BAM). Mean recovery time for KX was 32.8±7.1 min without antagonizing and 28.6±5.2 min following delivery of an antagonist. Mean recovery time was 6.2±0.8 min for BAM and 5.1±0.5 min for NalMed-A after antagonizing. Only with KX were raccoons observed to recover without use of an antagonist. Supplemental oxygen was provided to 23% (3/13), 72% (13/18), and 71% (12/17) of raccoons immobilized with KX, BAM, and NalMed-A, respectively. Hypoxemia at <80% oxygen saturation occurred in 0% (0/17), 27% (4/15), and 6% (1/16) of the raccoons administered KX, BAM, and NalMed-A, respectively; all raccoons fully recovered from chemical immobilization. All combinations could be used for raccoon immobilization; however, the need for delivery of supplemental oxygen to a majority of raccoons immobilized with BAM and NalMed-A may limit broader use of these agents for certain field studies involving capture, sample, and release of free-ranging animals from a practical standpoint.

Key words: Azaperone, butorphanol, ketamine, medetomidine, nalbuphine, raccoon, xylazine.

INTRODUCTION

Wildlife immobilization is recognized as an important tool for research and management. Chemical immobilization is one method that enables select procedures (e.g., surgery, blood collection) to occur safely for both the animal and researcher (Chinnadurai et al. 2016). Raccoons (*Procyon lotor*) are regularly handled in North America, particularly for disease research and management (Elmore et al. 2017). Raccoon rabies virus is enzootic throughout the eastern US (Ma et al. 2022) and has been a focus for national wildlife rabies control programs since the 1990s; the need to nonlethally capture, sample, and release raccoons for rabies management and research continues across many states (Gilbert and Chipman 2020). Chemical immobilization of raccoons in the context of wildlife rabies management includes surveys of population abundance (Slate et al. 2020), screening blood serum for evidence of rabies virus exposure (Bigler and Hoff 1974), measuring the impact of oral rabies vaccination management (Johnson et al. 2021), and related ecologic studies (Hill et al. 2023).

A commonly used raccoon immobilization drug combination is a 5:1 mixture of 20 mg/kg ketamine and 4 mg/kg xylazine (Kreeger and Arnemo 2018). Ketamine has a long history of

use as a wildlife anesthetic (e.g. Bigler and Hoff 1974) and has been used at varying dosages, such as 22–38 mg/kg (Belant 1995), 10 mg/kg (Deresienski and Rupprecht 1989), 5–15 mg/kg (Nielsen 1999), and in combination with other drugs such as acepromazine (Gehrt et al. 2001), and medetomidine (Robert et al. 2012). An alternative immobilization drug combination is tiletamine-zolazepam, with or without xylazine (Kreeger and Arnemo 2018). However, ketamine and tiletamine-zolazepam are US Drug Enforcement Administration (DEA) schedule III controlled substances, defined as drugs with a moderate to low potential for physical and psychologic dependence, subject to US federal regulations. Drug scheduling can change if the potential for human abuse changes (e.g., recent public safety alert concerning xylazine, an unscheduled substance; DEA 2023). Possession of controlled substances that may be popular for street abuse can present safety concerns for wildlife managers and researchers. When regulations or individual safety concerns may preclude the use of certain immobilization drugs, identifying alternative drug combinations may be necessary. An unscheduled drug that has been tested in raccoons is medetomidine, which is considered useful for brief noninvasive procedures (Baldwin et al. 2008). However, due to observations of shallow sedation and partial arousal at the dosage tested (0.21 mg/kg), alternative dosages or drugs may be needed for longer or more invasive procedures on raccoons.

Drug combinations may have synergistic interactions so that lower dosages of the parent drugs can produce similar or improved immobilization (Wolfe, Fisher, Davis et al. 2014). One example is butorphanol-azaperone-medetomidine (BAM), which is a combination of butorphanol (schedule IV) with azaperone and medetomidine (both unscheduled). This has been tested in several species, such as white-tailed deer (*Odocoileus virginianus;* Siegal-Willott et al. 2009), American black bears (*Ursus americanus*; Wolfe et al. 2008), lions, (*Panthera leo*; Semjonov et al. 2017), American bison, (*Bison bison*; Harms et al. 2018), rhesus monkeys (*Macaca mulatta*; Malinowski et al. 2019), and Asian palm civets (*Paradoxurus hermaphroditus*; Ahmad et al. 2021). Most studies noted that BAM was effective for immobilization; however, hypoxemia has been noted in guanaco (*Lama guanicoe*; Georoff et al. 2010) and red deer (*Cervus elaphus*; Wolfe, Fisher, Davis et al. 2014), and an insufficient depth of anesthesia was noted for hole drilling for tail transmitter placement in American beavers (*Castor canadensis*; Roug et al. 2018).

Nalbuphine-medetomidine-azaperone (NalMed-A) is an unscheduled drug combination that has been tested in multiple wildlife species, such as red deer, bighorn sheep (*Ovis canadensis*), cougar (*Puma concolor*), white-tailed deer (Wolfe, Lance, Smith et al. 2014), and recently raccoons (Doub et al. 2023). Successful immobilization was reported for most species; however, hypoxemia has been noted in bison (Wolfe et al. 2017), beavers (Roug et al. 2019), and black bears (Wolfe et al. 2016), and an insufficient depth of anesthesia was noted for hole drilling for tail transmitter placement in beavers (Roug et al. 2019).

Immobilization drugs may have various pharmacologic effects in different target species. Characterizing the effects of immobilization drugs on physiologic parameters and anesthetic depth in individual target species is necessary to determine safety, suitability, and practicality of the drugs for wildlife management and research activities requiring chemical immobilization of free-ranging wildlife. We conducted a trial in a controlled environment to compare two drug combinations, BAM and NalMed-A, for raccoon immobilization, to ketamine-xylazine (KX).

MATERIALS AND METHODS

Animals and housing

Raccoons were captured in the local vicinity of the National Wildlife Research Center (NWRC), Fort Collins, Colorado, US (40°35′03″N, 105° 08′50″W) during June and July 2017 for other

studies. The last use of immobilization drugs in this animal cohort had been 6 mo before the start of this study. Adult raccoons were housed in individual outdoor enclosures (3 × 3 × 2.5 m [width×length×height]) and following procedures approved by the NWRC Institutional Animal Care and Use Committee (QA-2856).

Immobilization and antagonist drugs

A 5:1 mixture of KX was prepared by adding 2 mL of xylazine (100 mg/mL, VetOne, MWI Veterinary Supply, Boise, Idaho, USA) to a 10-mL vial of ketamine (100 mg/mL, VetOne, MWI Veterinary Supply), resulting in 83.3 mg/mL ketamine and 16.7 mg/mL xylazine solution. Compounded BAM (27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, and 10.9 mg/mL medetomidine) and NalMed-A (40 mg/mL nalbuphine, 10 mg/mL azaperone, and 10 mg/mL medetomidine) were acquired in kits from Wildlife Pharmaceuticals (Windsor, Colorado, USA).

Yohimbine (2 mg/mL, Wildlife Pharmaceuticals) was administered at 0.11 mg/kg to antagonize the xylazine in KX. For BAM and NalMed-A, atipamezole (5.0 mg per 1.0 mg medetomidine, 25 mg/mL, Wildlife Pharmaceuticals) and naltrexone (0.233 mg/kg and 50 mg/mL, Wildlife Pharmaceuticals) were administered as the antagonists for medetomidine and for butorphanol or nalbuphine, respectively. Ketamine and azaperone have no known antagonists.

This study involved two stages. In stage 1, drug dosages were titrated. Crossover comparisons using the titrated dosages occurred during stage 2.

Stage 1: Drug dose titration

Raccoons were evenly distributed by weight into three groups: KX; BAM; and NalMed-A. Stage 1 was completed during 14 to 16 March 2018 using 17 raccoons: four females and three males in the KX group, four males in the BAM group, and three females and three males in the NalMed-A group. A step-up and step-down procedure was used, similar to the optimization procedure described by Ellis et al. (2019) in which we sequentially decreased the test dose by 20% following adequate immobilization or increased a given test dose by 20% following inadequate immobilization. The immobilization was graded as adequate (i.e., achieved level 4 induction and able to attach monitoring equipment) or inadequate (i.e., level 4 induction not achieved and unable to attach monitoring equipment). Once a drug dose resulted in adequate immobilization in two individual raccoons in succession, the drug was considered titrated for use in stage 2. Exceptions to our procedure due to events (e.g., low respiratory rate) not captured in grading the immobilization as adequate or inadequate are described in Supplementary Material (stage 1: drug dosage titration results). All drugs were administered intramuscularly with a hand syringe, with the raccoons manually restrained either using squeeze traps or within den boxes.

After administration of a drug combination, time to induction (levels 2–4) was recorded. Level 2 animals were ataxic and responsive to sound or nearby movement. Level 3 animals had head down and were responsive to touch or physical movement of the traps or den boxes. Level 4 animals were recumbent and unresponsive to touch (e.g., no blink reflex or ear twitch) or movement of the traps or den boxes. After observing level 4 induction, handling was initiated by removing the raccoon from the trap or den box for weighing. Animals were then positioned for vital signs monitoring, which included temperature, heart rate, respiratory rate, percentage of oxygen saturation (SpO_2), and blood pressure. A pulse oximeter (Rad-57, Masimo Corporation, Irvine, California, USA) was connected to the tongue to measure SpO_2 and an electronic sphygmomanometer (CONTEC08A-VET, Contec Medical Systems, Qinhuangdao, Hebei Province, China) with an inflatable cuff was placed on a forearm to measure blood pressure.

We assessed depth of sedation based on jaw tone, muscle rigidity, and responses to handling scored on a scale of 1 (tense, poor) to 5 (relaxed, good), following the recording of vital signs. Collection of vital signs and sedation depth occurred at approximately 5-min intervals until the animal appeared to be arousing (e.g., increased body tension or increased respiration) or until antagonists were administered. Respiratory rate monitoring continued during recovery, and raccoons were assessed for recovery at levels 2 to 4. At level 2, increased respiration was observed. At level 3, the animal's head was up. At level 4, the animal was ambulatory with or without stimulation (e.g., loud sounds or light touch with a Y pole).

Published parameters for raccoon vital signs are body temperature 37–40° C (98.6–104° F), respiratory rate 15–30 breaths/min, and heart rate 175–200 beats/min (Evans 2002). The SpO_2

ranges for raccoons were not located; however, for most animals, normal oxygen saturation ranges from 96% to 100% (Ayres 2012). Based upon veterinarian consultation and data from other species, an a priori acceptable range for immobilization to continue was determined; these parameters were SpO_2>80%, body temperature 36.1–40° C (97–104° F), and respiration rate ≥8 breaths/min. Vital signs at the ends of the published range and still within the a priori ranges were treated ad hoc, and immobilization was allowed to continue. Vitals outside the a priori range were treated immediately, with priority over continuing immobilization. Heart rate anomalies in the absence of other abnormal vital parameters did not constitute a reason for stopping the immobilization.

Treatment consisted of reducing body temperatures ≥39.0° C (102.2° F) by cooling with water and ice packs and warming for body temperatures ≤37° C (98.6° F) by covering with blankets; when SpO_2 was <90%, administering regulator-controlled (Regulator 290727-00, Airgas Puritan Medical, Inc., Radnor, Pennsylvania, USA) supplemental oxygen using an oropharyngeal catheter (J0656, Jorgensen Laboratories, Inc., Loveland, Colorado, USA) at 1–3 L/min. When vital signs were outside of the a priori ranges, we administered antagonists, especially when vital parameters were increasingly abnormal over time. Antagonizing KX was a limited treatment option, because antagonizing xylazine is not recommended for the first 30 min after the last injection to allow time for metabolism of the ketamine (Kreeger and Arnemo 2018).

Stage 2: Crossover comparisons

Eighteen (11 male and seven female) adult raccoons were categorized by weight (lightest six, 6.94–8.6 kg; middle six, 8.84–10.47 kg; and heaviest six, 10.48–12.65 kg) recorded on 6 March 2018 and randomly assigned into three groups (groups 1, 2, and 3). Three rounds of testing were completed with all raccoons being used once during each 3-d round. Each group was subdivided into two subgroups (A and B). Subgroup A did not receive an antagonist immediately after completing sample collection and was tested during the morning. Subgroup B received an antagonist immediately after completion of sample collection and was tested during the afternoon. The purpose of subgroup A was to ascertain how long immobilization and handling could safely continue in the absence of antagonists. During the initial round of stage 2, groups 1, 2, and 3 were assigned to receive BAM, NalMed-A, and KX, respectively. Assigned treatments for groups 1, 2, and 3 changed to KX, BAM, and NalMed-A, respectively, for round 2 and to NalMed-A, KX, and BAM, respectively, for round 3. Round 1 was completed during 25–27 April 2018, round 2 during 5–7 June 2018, and round 3 during 26 to 28 June 2018, providing the raccoons with at least a 2-wk rest between immobilizations.

Administration of chemical immobilization agents, vital sign monitoring, scoring sedation depth, interventive treatments, and recovery period monitoring occurred as described for stage 1. Two additional timed measurements (i.e., the time the animal was removed from the trap or den box and the time when animal handling started) were added to induction monitoring. Procedures for biologic sampling collection were added to the handling period and included collecting 3 mL of blood from the jugular vein, collecting two whiskers, pricking the gums with an 18-gauge needle (simulating tooth collection), and marking with an ear tag (1005-3, National Band and Tag Company, Newport, Kentucky, USA), placed between the frontal base and tip of the ear. The handling period ended when antagonists were given or when the animals appeared to be arousing (e.g., increased body tension or increased respiration), regardless of the status of sample collection. The recovery period began when the handling period ended and ended when the raccoon moved to resting areas within its enclosure.

Statistical analysis

Summary statistics were calculated for stages 1 and 2. In stage 1, we report the minimum and maximum vital sign values recorded during titration. In stage 2, we report on the vitals observed and the times associated with the different levels and phases of immobilization. Only the first vital signs recorded for each animal were used for the calculating treatment means. Unless noted, results are reported as mean±SE. Wilcoxon signed rank tests were conducted using R (R Core Team 2022) for comparing time to level 4 induction, time to handling start, and recovery period duration in stage 2. Times reported for recovery duration were analyzed, separated by

TABLE 1. Mean and SE of time in minutes along with the range and sample size to different levels of induction and recovery after administering the antagonists for ketamine-xylazine (KX), butorphanol-azaperone-medetomidine (BAM), or nalbuphine-azaperone-medetomidine (NalMed-A) to raccoons (*Procyon lotor*). The induction levels monitored included ataxia (level 2), head down (level 3), unresponsiveness (level 4), and also when the animal was removed from its trap and when handling started. The number of records (*n*) varied for different levels of induction and for recovery.

Drugs compared	Induction Level 2	Induction Level 3	Induction Level 4	Removed from trap	Handling start	Recovery after antagonizing
KX						
Mean (±SE)	2.8 (±0.3)	4.7 (±0.6)	7.7 (±0.7)	8.8 (±0.8)	10.0 (±0.7)	28.6 (±5.2)
Range	1.3–5.9	1.4–9.7	5.3–16.0	6.0–17.8	6.0–17.8	14.4–52.9
n	14	16	16	14	16	7
BAM						
Mean (±SE)	2.2 (±0.1)	3.3 (±0.2)	8.4 (±1.0)	9.4 (±1.0)	11.5 (±1.2)	6.2 (±0.8)
Range	1.3–2.7	2.1–4.9	3.4–18.5	5.7–22.0	6.3–22.0	2.5–16.0
n	16	18	18	16	18	18
NalMed-A						
Mean (±SE)	3.3 (±0.3)	4.5 (±0.5)	7.7 (±0.7)	11.5 (±1.0)	13.0 (±1.3)	5.13 (±0.5)
Range	1.6–6.0	2.2–9.5	4.2–14.0	6.2–18.8	6.5–27.8	2.5–9.5
n	14	17	17	16	17	17

whether the antagonist was or was not administered. Significance level was $\alpha \leq 0.05$, and a Bonferroni correction was applied by adjusting the significance level to $\alpha \leq 0.02$ to account for multiple comparisons (KX-BAM; KX–NalMed-A; and BAM–NalMed-A) for each immobilization phase.

RESULTS

Stage 1: Drug dose titration

All animals fully recovered from immobilization. Starting drug dosages were 10.0 mg/kg ketamine and 2.00 mg/kg xylazine (0.120 mL/kg KX), 0.546 mg/kg butorphanol, 0.182 mg/kg azaperone, and 0.218 mg/kg medetomidine (0.020 mL/kg BAM), and 0.800 mg/kg nalbuphine, 0.200 mg/kg azaperone, and 0.200 mg/kg medetomidine (0.020 mL/kg NalMed-A). Based on our drug titration process (see Supplementary Materials for stage 1 titration results, Tables S1 and S2, and Fig. S1), the titrated dosages used for stage 2 were 8.00 mg/kg ketamine and 1.60 mg/kg xylazine (0.096 mL/kg KX), 0.437 mg/kg butorphanol, 0.146 mg/kg azaperone, and 0.174 mg/kg medetomidine (0.016 mL/kg BAM), and 0.720 mg/kg nalbuphine, 0.180 mg/kg azaperone, and 0.180 mg/kg medetomidine (0.018 mL/kg NalMed-A).

Stage 2: Crossover comparisons

All 18 raccoons received each drug combination across the three different rounds of testing. Environmental ambient temperatures ranged from 7.5° C (45.5° F) to 38.3° C (101.0° F) during testing rounds (see Supplementary Material Table S3). The mean (±SE) of the actual dosages administered by volume were 0.104±0.007 mL/kg KX, 0.017±0.001 mL/kg BAM, and 0.020±0.001 mL/kg NalMed-A. This corresponded to specific mean dosages of 8.66 mg/kg ketamine and 1.74 mg/kg xylazine for KX, 0.464 mg/kg butorphanol, 0.155 mg/kg azaperone, and 0.185 mg/kg medetomidine for BAM, and 0.800 mg/kg nalbuphine, 0.200 mg/kg azaperone, and 0.200 mg/kg medetomidine for NalMed-A. Individual dosages are listed in the Supplementary Material (Table S4).

Five induction depth time points were recorded (Table 1). One raccoon that received two injections of KX to achieve immobilization was excluded from the induction analysis. Two

TABLE 2. The mean and SE along with the range and sample size associated with the first recording for body temperature, heart rate, and respiratory rate, and percentage of oxygen saturation (SpO_2) associated with ketamine-xylazine (KX), butorphanol-azaperone-medetomidine (BAM), nalbuphine-azaperone-medetomidine (NalMed-A) in raccoons (*Procyon lotor*) and prior to treatment with supplemental oxygen. The measurements occurred between 7 min and 29 min after administering the drug combinations.

Drugs compared	Temperature (C)	Heart rate (beats/min)	Respiratory rate (breaths/min)	SpO_2 (%)
KX				
Mean (±SE)	38.3 (±0.2)	71.9 (±3.7)	21.4 (±2.4)	91.6 (±0.7)
Range	36.7–39.8	48–100	9–41	84–96
n	18	18	18	17
BAM				
Mean (±SE)	38.2 (±0.3)	61.8 (±3.1)	7.7 (±0.4)	85.7 (±1.9)
Range	35.9–40.3	40–88	4–10	69–94
n	17	17	17	13
NalMed-A				
Mean (±SE)	38.5 (±0.3)	66.1 (±7.8)	8.5 (±0.7)	86.7 (±1.2)
Range	36.7–40.4	40–180	5–16	76–96
n	17	17	17	14

other raccoons (one given KX and one given NalMed-A) never reached a safe handling level and were excluded from the induction analysis and from the analyses of vitals and recovery. None of the pairwise comparisons were different from one another regarding duration of induction ($P>0.05$; Table S5).

Complete sample collection occurred for 89% (16/18), 72% (13/18), and 89% (16/18) of the raccoons administered KX, BAM, and NalMed-A, respectively. Partial sampling occurred in four raccoons due to stimulus-responsive behaviors (one each for KX, BAM, and NalMed-A) or because an antagonist was administered due to animal safety concerns (one BAM). Sampling was not performed on five raccoons: two animals (one KX one NalMed-A) with inadequate induction; and three animals (all BAM) antagonized prior to sample collection due to low respiratory rate (4 and 6 breaths/min; $n=2$) or a high body temperature (40.3° C [104.5° F]; $n=1$). For raccoons with completed sampling, handling times ranged from 6.8 min to 41.0 min for KX, 6.6 min to 107.2 min for BAM, and 7.7 min to 84.2 min for NalMed-A.

Mean body temperatures were consistent and within published normal ranges and the a priori range (37–40° C [98.6-104° F]) for all raccoons treated with the three drug combinations (Table 2). Mean heart rates using any of the three drug combinations were lower than the published range (175–200 beats/min). Mean respiratory rates were within normal (15–30 breaths/min) for KX and near or below the acceptable a priori range (>8 breaths/min) for BAM and NalMed-A. Mean SpO_2 was above 90% in KX-immobilized animals. Mean SpO_2 was below 90% but above 80% in animals administered BAM and NalMed-A. Mean blood pressure was not calculated due to difficulties collecting measurements. Detailed information on animal vital signs, including variability for each raccoon, are in the Supplementary Material (Table S6). Sedation depth was relaxed and good for immobilized raccoons (i.e., score of 5 across all measures): 87% (13/15) of KX; 88% (14/16) of BAM; and 94% (15/16) of NalMed-A. Three raccoons (two KX and one NalMed-A) with successful induction, but without any sedation depth scoring data due to responsive behaviors occurring after handling started, were excluded from the sedation depth summary proportions.

Oxygen was provided to 23% (3/13), 72% (13/18), and 71% (12/17) of raccoons administered KX, BAM, and NalMed-A, respectively,

based upon SpO_2 dropping below 90% or respiratory rate <15 breaths/min. Animals excluded from this assessment included four KX raccoons (oxygen information was not recorded) and animals that had inadequate induction (one KX and one NalMed-A). In animals with recorded SpO_2, values below 80% were observed in 0% (0/17), 27% (4/15), and 6% (1/16) of raccoons administered KX, BAM, and NalMed-A, respectively. Twenty-three raccoons (three KX, 10 BAM, and 10 NalMed-A) continued to have SpO_2 monitoring after receiving supplemental oxygen. After providing supplemental oxygen, SpO_2 below 80% continued in 20% (2/10) of the BAM-immobilized raccoons. A SpO_2 below 90%, but above 80%, was recorded in 40% (4/10) of the NalMed-A–immobilized raccoons. The SpO_2 for the remaining 17 raccoons reached 90% or higher following treatment with supplemental oxygen.

Antagonists were administered before the planned end of the handling period in 0% (0/17), 56% (10/18), 53% (9/17) of raccoons that received KX, BAM, and NalMed-A, respectively. A respiratory rate below 8 breaths/min (9/10 BAM; 7/9 NalMed-A) was the primary reason for antagonizing raccoons; others had a body temperature greater than 40° C (104° F) (1/10 BAM; 2/9 NalMed-A). Two of the raccoons antagonized due to a high body temperature were processed during ambient environmental temperatures of 38.3° C (100.9° F).

For KX, 10 raccoons recovered without using the antagonist, and seven raccoons were antagonized. All BAM (n=18) and NalMed-A (n=17) raccoons were administered antagonists; KX had the longest mean recovery period, which was 32.8±7.1 min without the antagonist and 28.6±5.2 min, following use of an antagonist. The mean recovery period for BAM- and NalMed-A–immobilized raccoons after antagonizing was 6.2±0.8 min and 5.1±0.5 min, respectfully (Table 1). Regardless of whether an antagonist was administered for KX, the recovery period was significantly longer ($P \leq 0.02$; Table S5) for KX-immobilized raccoons than for BAM- or NalMed-A–immobilized raccoons administered antagonists.

DISCUSSION

All raccoons recovered from the tested drug combinations, and sampling procedures could be completed for most animals administered each drug combination. Although the induction times were not significantly different among the drug combinations tested, KX had the shortest time to initiation of handling. Records from three raccoons immobilized with NalMed-A and two raccoons immobilized with BAM noted that the raccoons vocalized aggressively (i.e., snarling or growling) during attempts to remove them from traps and handle them. Such vocalizations were not reported for raccoons immobilized with KX. When indicators of inadequate immobilization (e.g., snarling, growling, or muscle rigidity) occurred after recording a level 4 induction, but before initiating handling for weighing and monitoring, the raccoon was left in place to provide more induction time; the times for "remove from trap" and "handling start" represent additional times when handling was reattempted.

Our observations related to BAM and NalMed-A induction in raccoons are consistent with reports for other species. Long induction times have been reported with NalMed-A (Wolfe et al. 2016) and are a recognized disadvantage of using BAM (Siegal-Willott et al. 2009; Kreeger and Arnemo 2018). Beavers immobilized with NalMed-A were left in traps for an additional 1–3 min after full induction was recorded before any attempts were made to handle animals (Roug et al. 2019). Also, vocalizations were reported in beavers administered NalMed-A (Roug et al. 2019). A higher dosage of NalMed-A (2.00 mg/kg nalbuphine, 0.500 mg/kg azaperone, and 0.500 mg/kg medetomidine for a 6-kg raccoon) shortened the induction time (mean of 6 min) for raccoons; however, one raccoon was noted to respond during handling and needed additional time for adequate immobilization, even using a higher dosage (Doub et al. 2023).

All drug combinations were associated with animal relaxation. Muscle tension was observed more often with KX. Sample collections occurred with all three drug combinations, but KX and NalMed-A had the highest proportions of raccoons with completed sample collection. In animals with stable vital signs, the handling period was less than 40 min for KX-immobilized raccoons, but both BAM and NalMed-A were observed to provide long handling periods (>1 h) for sample collection. Concerns regarding animal safety, however, often required that the duration of BAM and NalMed-A immobilization be intentionally shortened by administering antagonists.

All sampling procedures applied (venipuncture, ear tagging, or gum prick) occurred without issues across all three drug combinations. The gum prick procedure was used to approximate tooth extraction, because teeth have been extracted from raccoons for tetracycline biomarker analysis and age determination. However, it is unknown if the gum prick is a reasonable proxy for tooth extraction. Ketamine-xylazine has been used for venipuncture, ear tagging, and tooth extraction (Johnson et al. 2021). Venipuncture and ear tagging have been successful in other species immobilized with BAM or NalMed-A (Wolfe et al. 2008; Wolfe et al. 2017; Ellis et al. 2019) and specifically with raccoons immobilized with NalMed-A (Doub et al. 2023); however, published data were not located describing use of BAM or NalMed-A for tooth extraction. The suitability of BAM or NalMed-A to immobilize raccoons requiring a tooth extraction is still unknown.

Both BAM and NalMed-A elicited quick and smooth recoveries after administering the antagonists. Recovery time for BAM- and NalMed-A–immobilized raccoons was less than 10 min, except for one BAM raccoon that took 16 min. Our mean recovery time (5.13 min) for NalMed-A was slightly lower than that reported for raccoons given a higher dose (10 min; Doub et al. 2023). However, if raccoons were not given the antagonist, no signs of recovery were noted, even when the handling period continued for over 1 h. Some KX-immobilized raccoons were ataxic or exhibited twitching or shaking during recovery; this occurred with and without the administration of the antagonist. Drooling was occasionally observed in some KX-immobilized raccoons during the handling period and recovery but was not observed in BAM- or NalMed-A–immobilized raccoons. The shortest recovery time associated with KX was 14 min, while the longest was 85 min. The antagonist, when it was used, for KX was yohimbine administered intramuscularly; atipamezole may have use as an alternative (Janssen et al. 2017). Changes in delivery, dosage, or the antagonist used may impact the recovery time and should be considered in future research.

Hypoxemia was the primary concern for raccoons in all drug treatment groups, and supplemental oxygen was administered under all combinations based upon SpO_2 or respiratory rate. Both BAM and NalMed-A were associated with over 70% of the raccoons receiving oxygen supplementation, while less than 25% of KX-immobilized raccoons received oxygen supplementation. Vital signs for KX-immobilized raccoons remained stable within the a priori ranges for this study and did not meet critical thresholds for discontinuation. For raccoons immobilized with BAM and NalMed-A, over 50% of the immobilizations met critical thresholds for cessation, most often due to a low respiratory rate. Supplementary oxygen did not always alleviate apparent hypoxemia in immobilized raccoons; some individuals with low respiratory rates (<6 breaths/min) continued to have low (<80%) oxygen saturation, and for other individuals, oxygen saturation below the recommend 90% (Ayres 2012; Kreeger and Arnemo 2018) still occurred. Doub et al. (2023) did not use supplementary oxygen with NalMed-A–immobilized raccoons and reported a mean oxygen saturation below 90% and measured oxygen saturation below 80% at each time point, and even below 60% at one time point for at least one raccoon. Metrics not included in this study but that should be considered for future studies for evaluating animal safety are mucous membrane color, capillary refill time, and arterial oxygenation to better understand SpO_2 values and risk of

hypoxemia. Further research is also necessary to determine the level of risk and whether it can be easily mitigated using treatment with supplemental oxygen.

Raccoon immobilizations with NalMed-A and BAM were very similar, and both had the same advantage over KX of rapid recovery aided by the antagonists; NalMed-A is not a DEA-scheduled drug combination, providing a slight advantage over BAM, which is schedule IV. The raccoon snarls and growls observed in our study with NalMed-A and BAM did not result in any injuries to personnel or animals. However, the risk exists that handlers may react to the animal vocalizations in such a way that could cause injury to themselves or others. Alternatively, complacency to the vocalizations may result in misjudging the level of immobilization and increasing the risk of a bite injury. Higher drug dosages may reduce these risks to personnel; however, this may further exacerbate the negative impacts to the raccoon's health and safety during immobilization.

Overall, KX continues to show a robust safety margin for the health of the animal under immobilization and continues to be recommended for use with raccoons, if personnel safety concerns, scheduling regulations and restrictions, or extended animal recovery times do not preclude such use. Both NalMed-A and BAM were effective at immobilizing raccoons, but animal and personnel safety must be considered. Animals that do not respond to administration of supplemental oxygen should be administered the appropriate antagonist and recovered. Based upon our observations that animals did not spontaneously recover and remained immobilized until the antagonists were administered, animals immobilized with BAM and NalMed-A must be administered the appropriate antagonists.

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SUPPLEMENTARY MATERIAL

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