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Comparing Black Bear Immobilization Performance of Ketamine-Xylazine and Ketamine-Xylazine-Telazol®

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Running Head: Comparing black bear immobilization performance of ketamine-xylazine and ketamine-xylazine-Telazol®

Title: Comparing Performance of two anesthetic protocols in American black bears

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Abstract: Optimal wildlife anesthetic protocols should induce rapid immobilization, allow for rapid recovery, and provide a wide margin of safety. The ketamine and xylazine (KX) anesthetic protocol is commonly used in chemical immobilizations of *Ursus americanus* (American black bear); however, some biologists report unreliability due to inconsistent recovery times and side effects such as convulsions, sudden arousals, and hyperthermia. In recent years, some biologists have employed the ketamine, xylazine and Telazol® (TKX) anesthetic protocol, which requires relatively lower dose of ketamine and telazol when administered together. Black bears drastically decrease their vital and metabolic rates during hibernation as compared to the active and hyperphagic states. This scenario could alter the efficacy of anesthetic agents during different bear physiological stages. Unfortunately, there is no evidence on whether TKX performs better than the KX protocol under any bear physiological stage. Thus, our objective was to compare the chemical immobilization performance of both KX and TKX protocols in bears housed at Virginia Tech's Black Bear Research Center. Protocol performance was assessed through vital

sign frequencies, induction time, and recovery time in 16 bears (11F and 5M). We used linear models to compare variables across different metabolic states. We found more consistent vitals using TKX compared to the KX protocol. Induction times remained similar in pre-hibernation ~10min, yet TKX produced longer inductions (8min) than KX during hibernation, and TKX produced shorter inductions (21min) than KX post-hibernation. Route of reversal agent administration influenced recovery times regardless of anesthetic protocol or physiological stage, where intravenous was 21min faster than intramuscular, and 29min less than per rectum. Both TKX and KX produced immobilization lasting at least 100min. We recommend using KX if immobilizing bears during hibernation and administering TKX during the pre-hibernation and post-hibernation states, as it produces shorter induction times and less variable bear vital signs.

Introduction

An optimal anesthetic agent should induce rapid immobilization, provide a surgical plane of anesthesia, allow for rapid recovery, as well as a wide margin of safety (Cistola et al. 2004). The interactions of the drug combinations should allow for consistent measurements in the animal's vital signs to ensure that there are no dramatic changes that make it difficult for recovery. Inconsistency in effects occurring from drug treatment could negatively impact the animal and affect ability to work with the animal while anesthetized. The general goals of ethical humane treatment include limiting stress, distress, discomfort, and pain to the animals (Ellis et al. 2019). These factors must be considered when deciding which anesthetic protocol to apply. Safety of the animal handlers must also be taken into consideration. Through use of chemical immobilization agents there is improvement in efficiency for animal handlers as well as a decrease in stress induced physiological responses (Ellis et al. 2019). Consideration of the animals weight also plays a pivotal role in estimating the appropriate dosage in each particular

case. An over-dosage of a chemical immobilization agent can result in impaired physiological responses. Prolonged sedation or extensive recovery times are not favorable.

Black bears have been anesthetized using a variety of drug combinations such as Tiletamine-zolazepam, Medetomidine + Tiletamine-zolazepam, Xylazine + Tiletamine-zolazepam, Xylazine + Ketamine and oral carfentanil (Caulkett and Cattet 2002). In choosing a drug combination for anesthetizing black bears, it is important to recognize that they are monogastric and are prone to vomiting (Caulkett and Cattet 2002). Xylazine-Ketamine drug combination has been proven to be unreliable due to the inconsistent recovery times (Caulkett and Cattet 2002) . Xylazine-ketamine can put the animal at risk for convulsions and hyperthermia due to the high dose of ketamine that is required. The action of ketamine is not blocked by the reversal drug, yohimbine, which is able to significantly shorten the time to arousal, time to sternal recumbency and time to walking (Caulkett and Cattet 2002, Deresienski and Rupprecht 1989). Tiletamine-zolazepam drug combination has also been used in black bears and have resulted in reliable anesthesia and predictable signs of recovery (Caulkett and Cattet 2002). Tiletamine has also shown effects of erratic and prolonged recovery. Zolazepam is a benzodiazepine that induces sedation, however when large doses are administered re-sedation can occur. Xylazine is an α2-adrenergic agonist that induces sedation, visceral analgesia, and muscle relaxation. Xylazine can be reversed with an α_2-adrenergic receptor antagonist such as yohimbine. Ketamine is a cyclohexamine drug that induces rapid onset action, however results in adverse effects such as hyperthermia, muscle hypertonia and rigidity. This specific drug combination has minimal effects on the respiratory and cardiovascular systems, which allows for a high margin of safety (Caulkett and Cattet 2002).

Many different protocols have been used in black bear anesthesia, however the tiletamine, zolzepam, ketamine and xylazine drug combination has not been reported. Tiletamine, a cyclohexamine, elicits central nervous system dissociation, superficial anesthesia, and visceral analgesia (Ellis et al. 2019). Exploration of the TKX drug combination has become of interest to understand the overall effects on physiological responses and its overall effectiveness as an anesthetic agent. The TKX combination consists of two dissociative anesthetic agents and two analgesics (Williams et al. 2002). Through addition of ketamine and xylazine in place of diluent, anesthesia can be accomplished through use of a small volume. TKX requires lower doses due to synergistic mechanisms of these anesthetics. TKX is commonly used with the reversal drug, yohimbine which accelerates recovery through reversal of the sedative effects of xylazine (Williams et al. 2002).

Although TKX has not been reported in black bears the drug combination has been used in feral cats, pigs, sheep, and dogs. Feral cats were immobilized with 0.25 ml TKX (12.5 mg tiletamine, 12.5 mg zolazepam, 20 mg ketamine and 5 mg xylazine). These studies showed that although the combination is inexpensive and practical, some physiological responses were not favorable. Severe hypoxemia, which could result in organ impairment, was common among female cats. Hemoglobin saturation with oxygen was also below 90% which corresponded to clinical hypoxemia. This treatment also made it difficult to detect a pulse, due to the intense vasoconstriction that reduced the blood flow to many organs (Cistola et al. 2004). The use of TKX in feral cats resulted in a prolonged recovery. This drug combination was also used in camelids in addition to adding 2.5 ml of ketamine (250 mg) and 1 ml of xylazine (100 mg) into 500 mg of Telazol powder with final solution of 4 ml. This drug combination resulted in restraint for 40-60 minutes (HuiChu, 2014). It was observed that in commercial pigs the TKX drug

combination was given in a single intramuscular bolus injection. This technique was used to minimize the stress caused by restraint and to avoid the hyperkinetic reaction due to the injection. This technique also leads to more complete drug absorption, which ultimately allows for more central nervous system depression (HuiChu, 2014). Our goal was to assess the performance of the two anesthetic protocols, KX and TKX, on the vital signs heart rate, respiratory rate, rectal temperature, induction times and recovery times. Specifically, our two main objectives were: first, assess the performance of the two anesthetic protocols, KX and TKX, on the vital signs heart rate, respiratory rate, and rectal temperature; and second, comparing induction time and recovery times for both protocols. These variables were compared across different physiological states such as active, hibernation, and post hibernation.

Methods:

We performed 207 anesthetic procedures on sixteen American black bears (eleven females and five males) from October to May of each year to capture three major physiological states: hyperphagia (Pre-Hibernation), hibernating (Hibernation), and spring activity (Post-Hibernation). The black bears were captured by the Virginia Department of Game and Inland Fisheries (VDGIF) and housed at Virginia Tech's Black Bear Research Center (BBRC) between 2012 and 2016. The two anesthetic protocols, KX (Ketamine: 6.3 mg/kg + Xylazine: 1.3 mg/kg) or TKX (Telazol®: 1.6 mg/kg + Ketamine: 2.52 mg/kg + Xylazine: 1.5 mg/kg) were administered intramuscularly facilitated by an air pressurized dart-CO_2 pistol system (Dan-Inject, DanWild LLC, Austin, TX). The use of the reversal drug, yohimbine was administered (0.2-0.3 mg/kg) either intravenously, intramuscularly or per rectum. After anesthetic induction was achieved, vital signs were recorded every 5 minutes including heart and respiratory rates, and rectal temperature. All anesthetic data has been previously recorded between 2012 and 2016

under Institutional Animal Care and Use Committee at Virginia Tech protocols 12-112 and 15-162.

We constructed statistical analysis using variance (ANOVA) as pertinent for each of the variables ($\alpha = 0.05$). All statistical analyses were performed in R-Studio (R version 3.6.1, The R Foundation for Statistical Computing).

Results

Vital Signs

Rectal temperature resulted in significance across physiological stages ($F_{2,196}$=64.9; P<0.001) (Figure 1). Hibernation had the lowest temperature in both protocols, as expected ($\bar{x}$ KX =

= 34.8℃ vs. TKX = 35.4℃). Protocols KX or TKX were not significantly different ($\bar{x}$ KX=35.5℃ vs. TKX=36.2℃) ($F_{1,196}$=3.52; P=0.062) (Figure 1). The interaction between physiological stages and protocol were significantly different ($F_{2,196}$=8.01; P<0.01) (Figure 1). This interaction was driven by post hibernation states of the KX and TKX protocol –1.39±0.32℃ (P<0.001), indicating the average difference. The TKX protocol during post hibernation resulted in 1.4℃ higher rectal temperatures than the KX protocol. The interaction between physiological stages and protocol were not significant during pre-hibernation (KX-Pre-Hibernation vs TKX-Pre-Hibernation - $\bar{x}$ difference 0.06±0.19℃ (P=0.999)) or during hibernation (KX-Hibernation vs TKX-Hibernation – $\bar{x}$ difference 0.604±0.25℃ (P=0.164)). During hibernation in both protocols, there was a high degree of variability, shown by the values outside of the 95% confidence interval (Figure 1).

Heart rate was significantly different across physiological stages ($F_{2,198}$=18.34; P<0.001), with hibernation having the lowest heart rates in both protocols (figure 2). Protocol individually showed no significance ($F_{1,198}$=1.85; P=0.176), however the interaction between physiological status and protocol was highly significant ($F_{2,198}$=8.45; P<0.01). The TKX protocol had around 13.51 beats per minute higher than the KX protocol during post hibernation (KX-Post-Hibernation vs TKX-Post-Hibernation –13.51±3.49 bpm (P=0.002)). There was no significance in the protocols during pre-hibernation (KX-Pre-Hibernation vs TKX-Pre-Hibernation 2.79±2.08 bpm (P=0.762)) or hibernation (KX-Hibernation vs TKX-Hibernation –4.38±2.7 bpm (P=0.584)). During hibernation in both protocols, there was a high degree of variability, shown by the values outside of the 95% confidence interval. The KX protocol resulted in similar median values for hibernation and post hibernation.

Respiratory rates were significantly different across physiological stages ($F_{2,198}$=23.73; P<0.001), in which pre hibernation had the highest respiratory rates as expected (Figure 3). There were no differences between anesthetic protocols ($F_{1,198}$=2.95; P=0.087), however the interaction between physiological stages and protocol was significant ($F_{2,19}$=4.46; P=0.013) (Figure 3). The TKX protocol resulted in around 2.52 respirations per minute higher than KX during hibernation (KX-Hibernation vs TKX-Hibernation average difference -2.52±0.86 rpm (P=0.045)). During post hibernation, TKX also resulted in around 2.86 rpm than KX (KX-Post-Hibernation vs TKX-Post-Hibernation – average difference 2.86±1.12 rpm (P=0.012)). During hibernation in both protocols, there was a high degree of variability (Figure 3).

Anesthetic Timing

Induction times resulted in significance across physiological stages ($F_{2,190}$=13.82; P<0.001), in which hibernation had the longest induction times, as expected (Figure 4). During

hibernation, both protocols resulted in induction times around 10 minutes longer than pre hibernation (Hibernation vs. Pre-Hibernation 9.69±2.762 (P=<0.001)) (Figure 4). The interaction between physiological stages and protocol were also significant ($F_{2,190}$=7.59; P<0.001) driven marginally during hibernation (KX-Hibernation vs TKX-Hibernation - average difference -9.03±2.762 min (P=0.052)). During hibernation, the TKX protocol took around 9 minutes longer than KX, which was undesirable. There was no difference during the pre-hibernation (KX-Pre-Hibernation vs TKX-Pre-Hibernation 2.08±1.623 min (P=0.956)) or post hibernation physiological stages (KX-Post-Hibernation vs TKX-Post-Hibernation 10.08±3.603 min (P=0.126)). During hibernation in both protocols, there was a high degree of variability (Figure 4).

Recovery to head up with the use of yohimbine resulted in the significance in the route of administration ($F_{2,89}$=8.011; P =<0.001) (Figure 5). The intravenous route of administration w as 23.7 minutes less than intramuscular (P=0.00237) (Figure 5). The intravenous route was also ~20 minutes less than the per rectum route (P= 0.00315) (Figure 5). Per rectum was ~3 minutes l ess than intramuscular (P= 0.8161) (Figure 5).

Recovery of the bear to all four legs with the use of a reversal resulted in significance in the route of yohimbine administration ($F_{2,66}$=3.57; P=0.034) (Figure 6). The intravenous route of administration resulted to be around 28.7 minutes faster than per rectum (Per Rectum vs Intravenous 1.68± 31.4381 (P=0.038)) (Figure 6). There was no significance in protocol ($F_{1,66}$=0.1; P= 0.753) or physiological stages ($F_{2,66}$=1.63; P=0.203) individually (Figure 6). The interaction between protocol and physiological stages ($F_{2,66}$=0.59; P=0.559), protocol and route of yohimbine ($F_{2,66}$=0.53; P=0.594), physiological stages and route of yohimbine

($F_{4,66}$=0.7344; P=0.572), were not significantly different (Figure 6). Intravenous administration resulted in the quickest recovery times, followed by intramuscular and slowest was per rectum.

Recovery to head up without the use of a reversal resulted in the significance in the interaction between protocol and physiological status ($F_{1,60}$=4.818; P= 0.032) (Figure 7). The recovery to all four without the use of a reversal resulted in no significance in protocol ($F_{1,43}$=1.49; P=0.23), physiological stages ($F_{2,43}$=0.89; P=0.42), or the interaction between the two ($F_{1,43}$=0.01; P=0.94) (Figure 7). The average time to recovery for the KX protocol was 136 minutes, while the TKX protocols average time to recovery was 163 minutes (Figure 8).

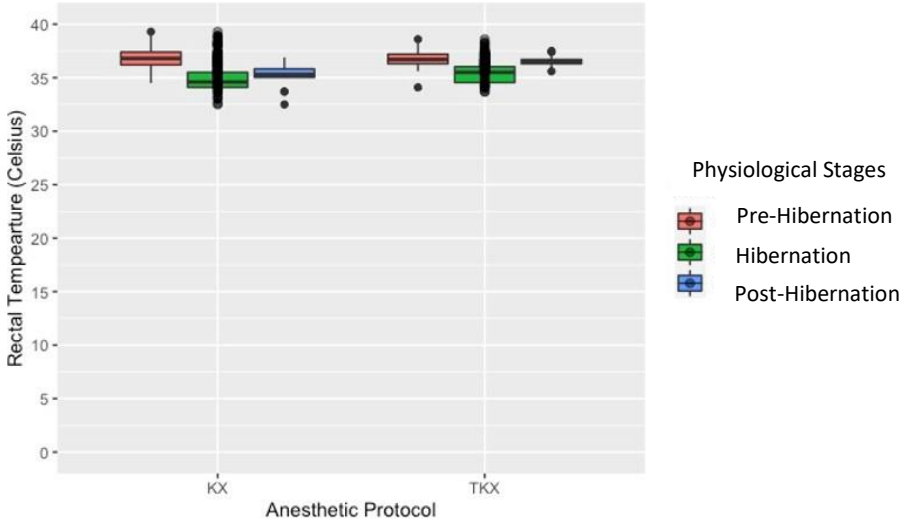

Figure 1. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on rectal temperatures (degrees celcius) during different physiological statuses (pre-habernation,hibernation and post-hibernation) in American black bears. The interaction between physiological stages and protocol was highly significant, with TKX resulting in higher rectal temperatures during post hibernation ($F_{2,196}$=8.01; P<0.01).

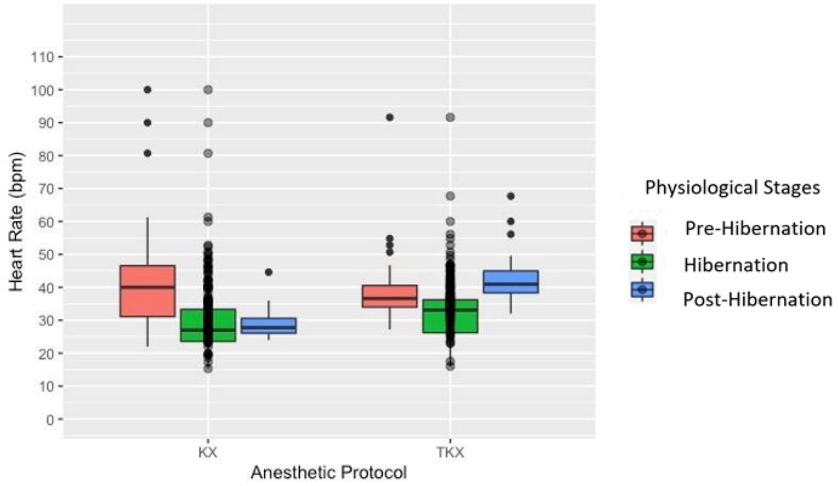

Figure 2. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on heart rate (beats per minute) during different physiological statuses (pre-habernation,hibernation and post-hibernation) in American black bears. During post hibernation, the TKX protocol resulted in higher heart rates than the KX protocol.

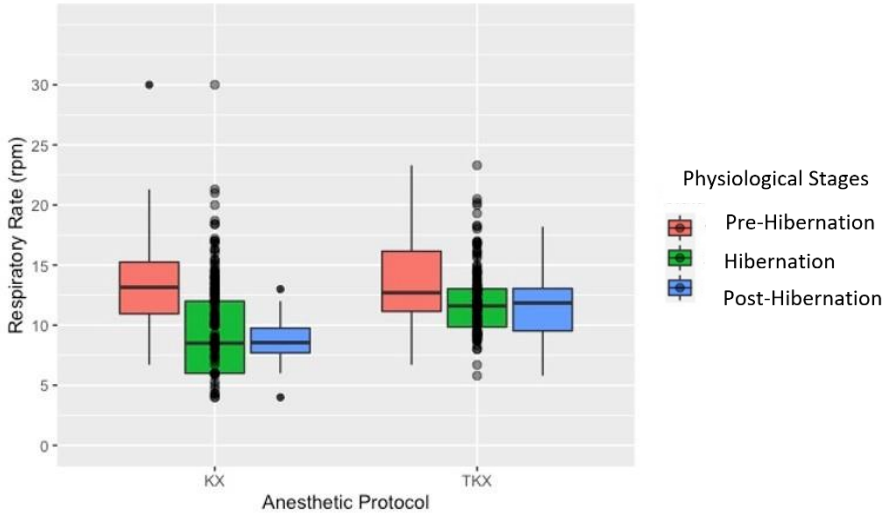

Figure 3. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on respiration rate (respirations per minute) during different physiological statuses (pre-habernation,hibernation and post-hibernation) in American black bears. During hibernation, TKX had slightly higher respiratory rates and significantly higher respiratory rates during post hibernation (KX-POST vs TKX-POST – average difference 2.86±1.12 rpm (P=0.012)).

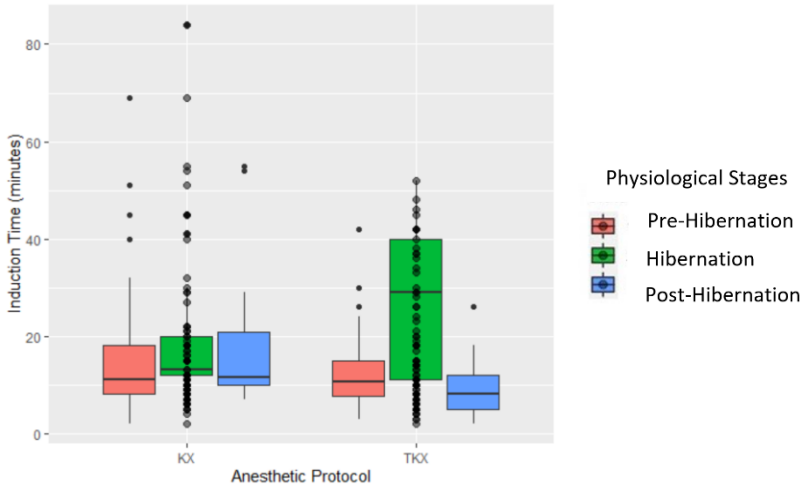

Figure 4. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on induction times (minutes) during different physiological statuses (pre-habernation,hibernation and post-hibernation) in American black bears.

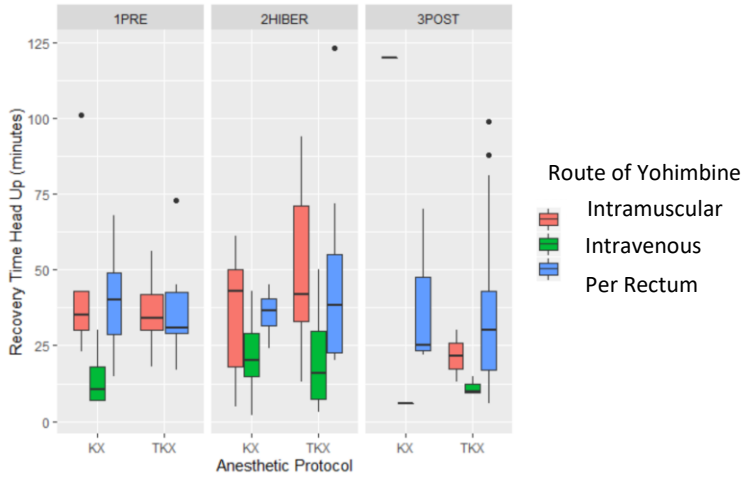

Figure 5. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on time to recovery of bear's head returning to an upward position (minutes) during different physiological statuses as well as the different routes of administration for yohimbine.

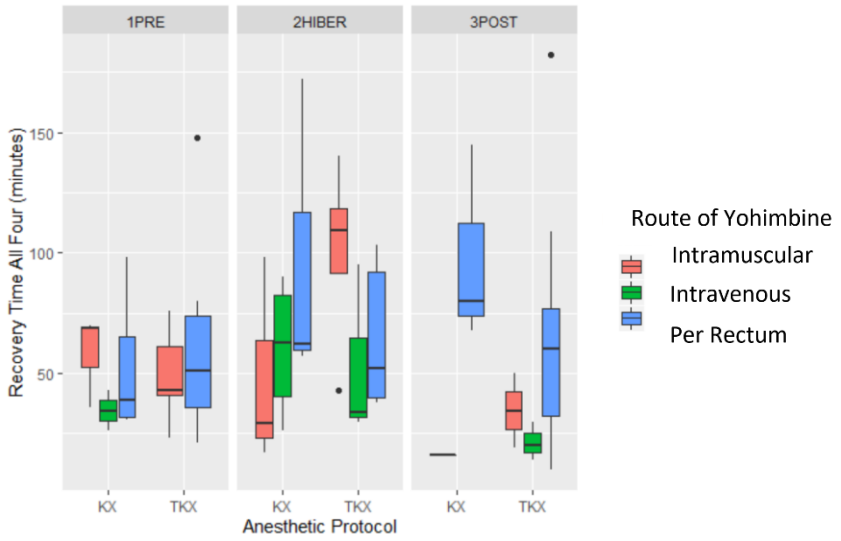

Figure 6. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on time to recovery of bears returning to all four legs (minutes) during different physiological statuses as well as the different routes of administration for yohimbine. The intravenous route of administration for yohimbine was the quickest, around 28.7 minutes less than per rectum.

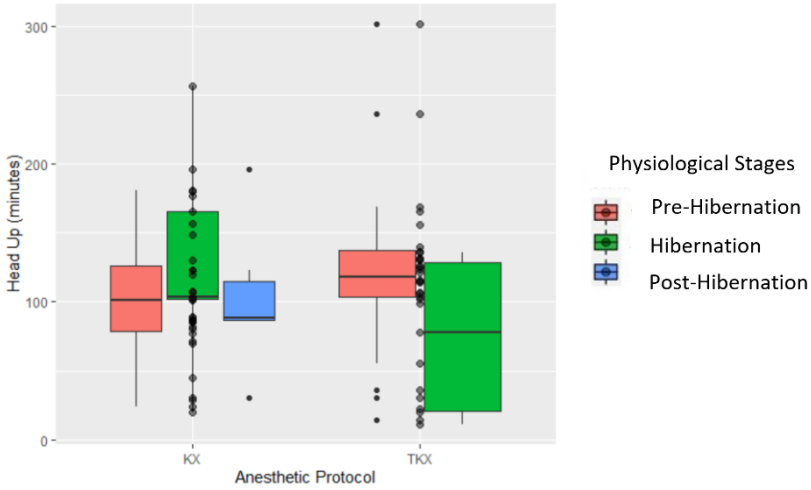

Figure 7. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on time to recovery of bear's head returning to an upward position (minutes) during different physiological statuses without the use of the reversal drug, yohimbine.

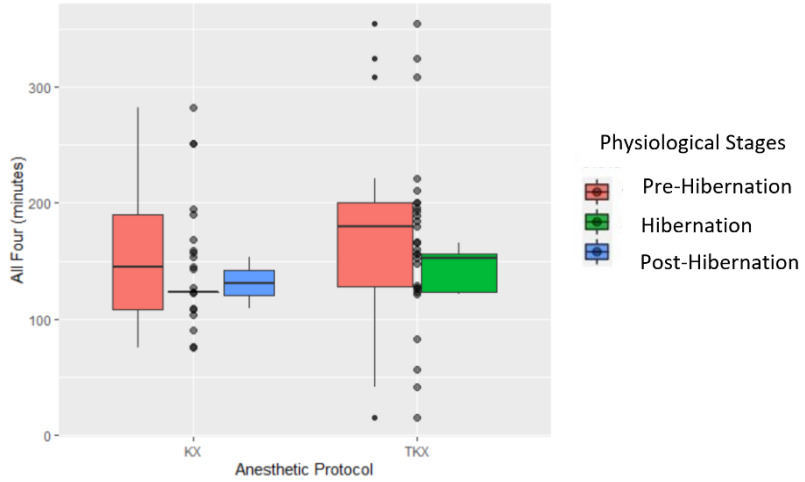

Figure 8. Box plot of the effects of anesthetic protocols (Ketamine + Xylazine or Telzaol + Ketamine + Xylazine) on time to recovery of bears returning to all four legs (minutes) during different physiological statuses without the use of the reversal drug, yohimbine. No significance in protocol or physiological stages. The average time to recovery for TKX was 163 minutes, and the average time to recovery for KX was 136 minutes.

Discussion

When comparing the anesthetic protocols KX and TKX vital signs and anesthetic parameters (induction time, recovery time and reversal time) provide insight on the influence of protocols during different physiological stages. Our results indicated that TKX influenced overall less variable vital signs than KX, which has been previously reported in black bears (Caulkett and Cattet 2002). Rectal temperatures, heart rate, and respiration rates were all less variable when using the TKX protocol.

Additionally, bears anesthetized using the TKX protocol exhibited significantly higher vital signs during post hibernation (Figures 1-3). The TKX protocol allowed for higher vitals post hibernation because this is representative of the bear being able to recover from anesthesia.

It is important for the bears to be able to return to their increased metabolic rates during post hibernation. The similar vital signs during hibernation and post hibernation when using the KX protocol can be explained by the KX drug combination causing deeper anesthesia by increased suppression of their metabolic rates. Those bears could be experiencing a deeper anesthesia unable to return to their expected vital rates or may have an increased sensitivity to KX.

The physiological stage, hibernation, produced the most variability across all vital signs (Figures 1-3). During hibernation, there were many data points that were outside of the 95% confidence interval. This high degree of variability could be explained by hibernation/arousal cycles. During hibernation, bears experiences cycles of depressed metabolic rates that fluctuate every 1.6-7.3 days (Tøien and Barnes 2015). When we anesthetized the bears during hibernation, the point in their hibernation cycle as undetermined. Some bears may have been anesthetized when their metabolic rates were depressed while others were not. Due to this initial variability in physiological state, there can be a higher degree of variability in vitals during hibernation when anesthetized using either protocols. Although the mechanism of function behind these cycles are unknown, shorter cycles could predict higher rates of heat loss due to colder conditions (Tøien and Barnes 2015).

The TKX protocol also resulted in undesirably longer induction times during hibernation. These induction times were around 50% longer than when using the KX protocol, shown in figure 4. These undesirable induction times could be caused by the lower doses that were administered of each drug when using the TKX protocol. When using the TKX protocol, 1.5 mg/kg of each drug were administered, which reduced the amount of ketamine by around four times. Due to the decreased doses, these bears may have been less able to cause induction.

Based on our results, the use of the reversal drug, yohimbine, decreased recovery times significantly, both head up and all four, shown in figures 5 and 6. When comparing recovery tim es of bears returning to all four legs (figure 6 and 8), the time to recovery was significantly less when using yohimbine. The use of intravenous administration was significantly quicker (around 28.7 minutes) than per rectum and intramuscularly. Intravenous administration is able to be distri buted faster in the central nervous system in comparison to the other routes of administration.

Conclusion

We recommend using the TKX protocol during pre and post hibernation physiological statuses. TKX during pre and post hibernation produced smaller induction times and lower variability in vital signs. TKX also provided more consistency in vitals which is important in optimal performance of anesthetic protocols. However, during hibernation we recommend using the KX protocol because of the shorter induction times. Further research on this data could include oxygen saturation as a measurement of blood oxygenation.

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