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New chemical restraint protocol in *Alouatta caraya* in captivity

Novo protocolo de contenção química em *Alouatta caraya* em cativeiro

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ABSTRACT: The black howler (*Alouatta caraya*) is an excellent study model for its similarity to anthropoid primates, including humans. Therefore, studies on the chemical restraint of these animals is essential. The objective of the present study is to evaluate the quality of sedation and anesthetic recovery in the chemical restraint of *A. caraya* under human care. Six adult animals were used, being three males (7.5±1.24 kg) and three females (4.53±0.40 kg). All were healthy *A. caraya* residing at the Brazilian Primate Center (CENP), where the combination of ketamine hydrochloride (7.5 mg/kg), midazolam (0.3 mg/kg), and dexmedetomidine hydrochloride (0.015 mg/kg) was tested, followed by reversal with atipamezole (0.03 mg/kg). The heart rate (HR), respiratory rate (RR), internal temperature (IT), SpO2, systolic and diastolic blood pressures (SBP/DBP), and mean arterial pressure (MAP) parameters were evaluated at three different times, and glycemia at two different times, during a 30 minute period, in addition to recovery status. Statistical variation was observed in HR, IT, and glycemia, which reduced over the observed time, but without losses that required intervention during the procedure. The recovery was smooth and the animals returned to their original enclosures without incident. The use of this protocol is recommended for chemical restraint of captive *A. caraya.*

Keywords: dexmedetomidine; midazolam; ketamine; chemical restraint; primate.

RESUMO:O Bugio-preto (*Alouatta caraya*) é um excelente modelo de estudo pela sua similaridade com os primatas antropoides, incluindo os humanos. Sendo assim, estudos sobre a contenção química desses animais é essencial. Objetivou-se com este estudo avaliar a qualidade de sedação e retorno anestésico na contenção química de *A. caraya* sob cuidados humanos. Foram utilizados seis animais adultos, sendo três machos (7,5±1,24 kg) e três fêmeas (4,53±0,40 kg), hígidos de *A. caraya* residentes no Centro Nacional de Primatas (CENP), onde testou-se a associação de cloridrato de cetamina (7,5 mg/kg), midazolam (0,3 mg/kg) e cloridrato de dexmedetomidina (0,015 mg/kg), e posterior reversão com atipamezole (0,03 mg/kg). Foram avaliados os parâmetros FC, FR, TI, SpO2, PAS, PAD e PAM em três diferentes momentos, e a glicemia em dois momentos, durante 30 minutos, além da recuperação em escores. Observou-se variação estatística em FC, TI e glicemia que reduziram ao longo do tempo observado, mas sem prejuízos que exigissem intervenção durante o procedimento. A recuperação ocorreu de forma tranquila e os animais retornaram sem intercorrências aos recintos de origem. Recomenda-se o uso deste protocolo para contenção química de *A. caraya* de cativeiro.

Palavras-chave: dexmedetomidina; midazolam; cetamina; contenção química; primata.

INTRODUCTION

The black howler monkey (*Alouatta caraya*) belongs to the Atelidae family and is one of the species with the widest geographic distribution within its genus. It is found in the Brazilian Amazon, Brazilian savanna (Cerrado), and Brazilian wetlands (Pantanal), as well as parts of Paraguay and the northern and northeastern regions of Argentina (IUCN, 2022; Neville *et al.*, 1988). It is one of the few genera that exhibit sexual dimorphism, with males being black and females displaying yellowish-brown fur. They are known as a vocalizing species, using their calls to defend their territory (IUCN, 2022; Nascimento *et al.*, 2007; Neville *et al.*, 1998). It is considered a species vulnerable to extinction, according to the list of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, due to the great anthropogenic pressure on its habitat (Verona; Pissinati, 2014).

A. caraya is an excellent study model for its similarity to higher primates, including humans. Furthermore, they are widely used as an experimental model in studies of infectious diseases such as yellow fever and malaria, as well as in understanding the transmission of diseases between primates, humans, and domestic animals, aiming to contribute to the surveillance and prevention of wild epizootics (Kowalewski *et al.*, 2011). Further studies are urgently needed in order to provide clinically relevant and species-specific information, as well as its sanitary status (Yarto-Jaramillo *et al.*, 2022).

Normally, when handling wild animals, anesthetic combinations are used for chemical restraint or even anesthesia, aiming at greater safety for the animal and the handlers (Diniz, 1997). Such combinations are used to seek pharmacological synergism, reduce doses and adverse effects, and enhance the individual sedative effects of each

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substance. Ketamine is the most commonly used agent in primates for chemical restraint, normally associated with alpha 2-adrenergic agonists and/or benzodiazepines (Caulkett; Arnemo, 2017).

When sedating captive primates, detailed monitoring and/or follow-up during the recovery from anesthesia is not always possible. Thus, studies aimed at developing a potentially reversible anesthetic combination that ensures a smoother and faster recovery are important and desirable (Freitas, 2019). The aim of the present study was to evaluate the quality of sedation and anesthetic recovery in the chemical restraint of captive A. caraya, aiming at performing minimally invasive and short-duration outpatient procedures.

MATERIAL AND METHODS

The present study was approved by the Animal Experimentation Ethics Committee (CEUA/IEC) certificate nº26/2022, and authorized by the Biodiversity Authorization and Information System (SISBIO) under protocol 86066-1.

Six adult animals aged between one and three years old were used, being three males (7.5±1.24 kg) and three females (4.53±0.40 kg), healthy,belonging to the Brazilian Primate Center (CENP), located on highway BR 316 KM 7, Ananindeua, Pará, Brazil.

The animals were kept in a cage with a movable press wall measuring 1 meter in length, 1.5 meters in width, and 1 meter in height, as only animals considered healthy were used.

The enclosures were cleaned daily by changing the wood shavings substrate and a diet was provided once a day, consisting of super-premium extruded feed for non-ruminant herbivorous primates, foliage, fruits, eggs, and water ad libitum.

For the experiment, all animals were on a twelve-hour food and six-hour water fast. The calculation of the volume of the drugs was performed according to the clinical record of the last weighing of the animals, and after five minutes of application and permission of the animal to be handled, they were weighed again to confirm the current real weight. There was no need for dosage adjustments, and no additional doses were administered throughout the experiment.

Drugs

The protocol was defined based on doses of drugs from the same classes used in the genus *Alouatta* and similar species, consisting in the combination of ketamine hydrochloride (7.5 mg/kg), midazolam (0.3 mg/kg), and dexmedetomidine hydrochloride (0.015 mg/kg), injected in the same syringe into the quadriceps femoris muscles. For reversal, atipamezole (0.03 mg/kg) was used, also intramuscularly (Benarrós, 2024).

Monitoring

Five minutes after the administration of the drugs, physiological parameters were recorded at three distinct time points (M0, M1, and M2), with each time point evaluated every 10 minutes. Then, after 30 minutes, the reverser was applied and the recovery phase was observed until the animal returned to its original enclosure.

The following physiological parameters were monitored: heart rate (HR)—in beats per minute (BPM)— obtained by the time interval between two consecutive R-wave intervals in the electrocardiographic tracing; systolic (SBP) and diastolic (DBP) blood pressure—in mmHg— collected with a cuff sized equivalent to 40% of the limb circumference and placed above the left talocrural joint; partial oxyhemoglobin saturation (SpO2)—in %— measured with a sensor on the first finger of the right pelvic limb; and internal temperature (IT), in degrees Celsius, through a rectal sensor. All readings were observed on a portable multiparameter monitor R1000VET® (RZ Vet, São Paulo, Brazil).

Respiratory rate (RR), through thoracoabdominal movements over a one-minute interval was also observed, and blood glucose levels were measured in mmol/L using a glucometer (G-Tech Free®, Accumed, Rio de Janeiro, Brazil) through a drop of blood obtained by pricking the right-hand index finger, but only at M0 and M2.

Simultaneously, the quality of immobilization, analgesia, and muscle relaxation was evaluated according to the protocol by Morin And Berteaux (2003), which categorizes the responses as follows: intense (perfect handling of the animal without complications and/or responsive movements), intermediate (slight response to stimuli such as electrode placement, blood collection, or pinching), and superficial (limb or head movements, vocalization, and difficulties in handling).

Recovery

After 30 minutes of drug application, the animal was placed in a calm environment inside a containment cage for the application of the reverser (atipamezole) and evaluation of recovery from the sedative effect.

The recovery phases were evaluated according to the following scores: I– 30 minutes before reversal, II– 5 minutes after reversal, III– 10 minutes after reversal, IV– attempts to sit, and V– attempts to stay in station (Benarrós, 2024). The observation of recovery ended when the animal was standing normally, with full restoration of behavior as observed before chemical restraint.

Statistics

Generalized linear mixed models (GLMMs) were used to quantify the influence of time since the start of the anesthetic protocol, the weight, and sex of the individuals on the different parameters (HR, RR, TI, SPO2, SBP, DBP, and glucose), considering $p<0.05$.

These models combine the properties of linear mixed models, which incorporate random effects to quantify the variation between sampling units, and generalized linear models, which use link functions and exponential distribution families to deal with data that do not have a normal distribution (Bolker *et al.*, 2009). Given that the study includes non-negative metrics, we assumed a Gaussian distribution with a logarithmic link function to generate the models and considered individuals as random effects added to the model. The analysis was performed in an R Core Team (2020), through the lme4 package (Bates *et al.*, 2015).

RESULTS AND DISCUSSION

There were no anesthetic complications during the

analyzed period, with an average latency period of 5±2.96 minutes, and the quality of immobilization and analgesia considered intense throughout the monitoring.

The statistical results of the evaluated parameters indicate that in M2 there was a significant variation in HR, suggesting that, even keeping all other variables constant, an increase in time is associated with a decrease in heart rate. Significant effects on IT were also noted at M2, where, as time increased, it tended to decrease, suggesting an inverse relationship between time and the reduction in IT. Finally, as time increased, glucose also tended to decrease (Tables 1, 2, and 3).

The other parameters of HR, SpO2, SBP, and DBP did not demonstrate significant variation in relation to time, nor was there any relevant variation in relation to weight and sex, with the values measured remaining within normal limits during the 30 minutes observed (Tables 1, 2, and 3).

Fagundes and collaborators (2020) also observed a reduction in IT and HR in *Alouatta guariba* sedated with a combination of dexmedetomidine, midazolam, and butorphanol, in addition to a reduction in RR, MAP, SpO2, and arterial lactate, demonstrating that the presence of a dissociative drug can contribute to less depression of the respiratory system due to its potential bronchodilator effect.

In contrast, Santos and collaborators (2017) did not notice relevant statistical variations in the use of ketamine and midazolam as pre-anesthetic medication, in doses similar to those in the present study for the digit amputation procedure in *A. guariba* in any of the physiological parameters, with only a slight reduction in temperature being observed. Similarly, Minto and collaborators (2021) also used this protocol prior to fracture repair surgery in the radius of *A. caraya*, also without significant physiological changes.

It is important to note that in both studies mentioned, the use of general anesthetic was necessary to enable the procedures. The combination of more potent and selective muscle relaxants, such as dexmedetomidine, allows for a reduction in the requirement and use of general anesthetics, which are known to affect blood pressure and hemodynamics over time.

It is also emphasized that, even in the presence of statistically significant changes, the values of the physiological parameters in the present study did not show clinically relevant alterations that would justify any intervention, remaining within the expected range for the species under sedation.

Freitas (2019) conducted a comparative analysis of the combination of ketamine with dexmedetomidine and with midazolam separately, finding results similar to those of the present study, being a reduction in cardiovascular parameters with the use of dexmedetomidine. However, muscle relaxation was more pronounced, making its use more favorable compared to the group with midazolam. The intense muscle relaxation caused by alpha-2 agonists is particularly useful in studies involving large and highly dangerous animals.

Unlike what is more commonly observed with the use of dexmedetomidine, due to its influence on insulin regulation and release, in the present study, a reduction in blood glucose levels over time was observed, similar to the results found in *Sapajus apella* by Benarrós and colleagues (2024). The values did not indicate severe hypoglycemia that would require intervention during the procedure. However, it is important to note that if this protocol is used in juveniles or animals with greater sensitivity to hypoglycemic crises, further studies are recommended to better understand this potential adverse effect in non-human primates.

Recovery was evaluated using scores, number of attempts by the animal until fully standing, and the possibility of returning to the original enclosures. The average time for complete recovery, after the administration of atipamezole and following 30 minutes of observation, was 30.6 ± 10.19 minutes. All the animals were still asleep, and were placed in a restraint cage for the administration of the reversal agent and observation of the parameters (Table 4). There were no complications during recovery and the animals returned with the normal behavior of the species to the enclosures of origin.

Regarding recovery time, few authors describe

Table 1 – Mean and standard deviation of physiological parameters at each evaluation time of the dexmedetomidine, ketamine, and midazolam protocol in males (M) and females (F) of *Alouatta caraya*. bpm- beats per minute; brpm- breaths per minute; ºC-degrees Celsius; mmHg-millimeters of mercury; mmol/L-millimoles per liter. Lowercase letters demonstrate the parameters that have undergone statistical variation among themselves over time.

Moments	M0		M1		M2	
Parameters/Sex	M (n=3)	F (n=3)	M (n=3)	F (n=3)	M (n=3)	F (n=3)
HR (bpm)	127.6±14	135±29.4	122.3±18.2	122±26.5	117.6±19.6	115.6±20.5
RR (brpm)	16.6±5.7	20±4	14±3.4	21.3±6.1	12±3.4	29.3±16.1
IT (°C)	35.8±0.8	36.7±0.8	36.2±0.9	36.6±0.6	35.8±0.3	36.4±0.5
SpO2 (%)	100±0	92.3±5.8	99.6±0.5	96.6±2.8	100±0	95±7.8
SBP (mmHg)	126±9.8	126.6±36.8	127.3±35.4	118.6±18.7	128.3±15.5	143.3±41.7
DBP (mmHg)	64.6±10	88.3±46.5	88±53.7	80±34.3	87.3±19.1	105±43
Glucose (mmol/L)	109.3±62.6	214±95.7	-	-	139±77.1	176.3±47

Table 2 – Influence of time on parameters in GLMMs. Significance levels: 0 "0.001" 0.01 " 0.05 '.'0.1 '' 1 (***p < 0.001, **p < 0.01, *p < 0.05).

GLMM Effects	Coefficient	Standard Error (Coefficient)	T-Value	P-Value
HR				
Intercept	5.286	0.161	32.775	<0.001***
Time 1	-0.070	0.068	-1.037	0.300
Time 2	-0.121	0.069	-1.742	0.081
Time 3	-0.208	0.092	-2.250	0.024*
RR				
Intercept	3.230	0.578	5.591	<0.001***
Time 1	-0.003	0.207	-0.016	0.987
Time 2	0.211	0.191	1.103	0.270
Time 3	0.002	0.275	0.006	0.995
IT				
Intercept	3.554	0.041	85.842	<0.001***
Time 1	0.004	0.004	0.928	0.354
Time 2	-0.006	0.004	-1.463	0.144
Time 3	-0.022	0.005	-4.290	<0.001***
SPO2				
Intercept	4.505	0.045	99.225	<0.001***
Time 1	0.020	0.020	1.001	0.317
Time 2	0.013	0.020	0.673	0.501
Time 3	-0.004	0.025	-0.147	0.883
SBP				
Intercept	4.860	0.264	18.384	<0.001***
Time 1	-0.028	0.115	-0.243	0.808
Time 2	0.074	0.109	0.678	0.498
Time 3	0.153	0.129	1.187	0.235
DBP				
Intercept	4.860	0.264	18.384	<0.001***
Time 1	-0.028	0.115	-0.243	0.808
Time 2	0.074	0.109	0.678	0.498
Time 3	0.153	0.129	1.187	0.235
Glucose				
Intercept	6.986	0.986	7086.000	<0.001***
Time 2	-0.090	0.008	-1.106	<0.001***

the use of reversal agents in non-human primates. However, a faster and more satisfactory recovery with the use of dexmedetomidine, compared to ketamine and midazolam, has been described for *A. guariba*, which reduces the anesthetic risk and the time the animal will be kept away from its original social group (Fagundes, 2020).

It is also important to emphasize that the recovery becomes more efficient when the specific antagonist of the drug is used, as in the case of this study, reducing potential side effects at this time. Monteiro and collaborators (2018) observed the presence of sialorrhea and a longer recovery time with the use of yohimbine over atipamezole in *A. guariba* anesthetized with ketamine, methadone, and dexmedetomidine, reinforcing the importance and benefit of using atipamezole.

CONCLUSION

It is concluded that the protocol composed of dexmedetomidine, ketamine, and midazolam and reversal with atipamezole is viable for the chemical restraint of captive adult *A. caraya*, without major hemodynamic damage, for performing minimally

Table 3 – Influence of weight and sex in parameters in GLMM. Significance levels: 0 "0.001" 0.01 " 0.05 '.'0.1 '' 1 (***p < 0.001, **p < 0.01, *p < 0.05).

GLMM Effects	Coefficient	Standard Error (Coefficient)	T-Value	P-Value
HR				
Weight	-0.089	0.033	-2.692	0.007**
Sex	0.256	0.103	2.471	0.014*
RR				
Weight	-0.028	0.124	-0.226	0.821
Sex	-0.493	0.356	-1.385	0.166
IT				
Weight	0.010	0.009	1.122	0.262
Sex	-0.046	0.030	-1.539	0.124
SPO2				
Weight	0.007	0.009	0.740	0.459
Sex	0.040	0.031	1.276	0.202
SBP				
Weight	0.001	0.054	0.023	0.982
Sex	-0.059	0.178	-0.333	0.739
DBP				
Weight	0.001	0.054	0.023	0.982
Sex	-0.059	0.178	-0.333	0.739
Glucose				
Weight	-0.378	0.212	-1.784	0.075
Sex	0.562	0.711	0.791	0.429

Table 4 – Evaluation of scores and attempts at recovery of *Alouatta caraya* after use of dexmedetomidine, ketamine, and midazolam and reversal with atipamezole.

Stages	Scores (X% of animals observed)	Attempts (number of attempts per X% of animals)
I (30 min before reversal)	1 (calm) - 100%	-
	3 (active) - 0%	
	7 (excited) - 0%	
	10 (uncontrollable) - 0%	
II (5 min after reversal)	1 (calm, occasional effort) - 100%	-
	3 (active) - 0%	
	5 (struggling) - 0%	
III (10 minutes after reversal)	1 (calm) - 100%	-
	3 (active) - 0%	
	5 (excited) - 0%	
	10 (struggling and with falls) - 0%	
IV (Number of attempts to sit)	-	1 - 33% (2 animals)
		2 - 50% (3 animals)
		4 - 17% (1 animal)
V (Number of attempts to stand)	-	1 - 50% (3 animals)
		2 - 16.6 % (1 animal)
		3 - 16.6% (1 animal)
		5 – 16.6% (1 animal)

invasive outpatient procedures.

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