ORIGINAL ARTICLE

Evaluation of blood pressure in feline night monkeys (*Aotus azarae infulatus*) under different restraint protocols

Rosa Helena de Figueiredo Chaves[1], Nazaré Fonseca de Souza[1], Aline Amaral Imbeloni[2], Ana Cláudia de Souza Neves[1], Renan Kleber Costa Teixeira[3] & Camila de Cassia dos Santos[1]

1 Universidade Federal Rural da Amazônia (UFRA), Instituto da Saúde e Produção Animal (ISPA), Pará, Brazil
2 Centro Nacional de Primatas (CENP), Secretaria de Vigilância em Saúde (SVS), Ministério da Saúde(MS), Ananindeua, Pará, Brazil
3 Universidade do Estado do Pará (UEPA), Centro de Ciências Biológicas e da Saúde (CCBS) Laboratório de Cirurgia Experimental (LCE), Belém, Pará,Brazil

Keywords
Aotus – anesthesia – oscillometric method – physical restraint

Correspondence
Rosa Helena de Figueiredo Chaves, Universidade Federal Rural da Amazônia (UFRA), Instituto da Saúde e Produção Animal (ISPA), Avenida Presidente Tancredo Neves, N° 2501, 66077-830, Belém, Pará, Brazil.
Tel.: +55 91 988961978 / +55 91 32545657;
fax: +55 91 3274-3814;
e-mails: rosinhahelena@gmail.com, rosa.chaves@ufra.edu.br

Accepted July 2, 2015.

Abstract

Background Feline night monkeys are very susceptible to stress when handled, and because of that, the use of anesthetic and physical restraint methods should be carefully evaluated. To ensure a safer anesthesia to the animals the present study aimed to observe the influence of four restraint protocols in the blood pressure of Aotus azarae infulatus.
Methods Blood pressure was obtained from 10 animals subjected to restraining protocols using tiletamine and zolazepam, isoflurane, ketamine and midazolam, and physical restraint and results were evaluated to determine their effect on this exam.
Results Among the restraint protocols tested, the anesthetic isoflurane promoted lower blood pressure values and no differences were observed between values measured in both arms in all groups.
Conclusion The results of our study suggest that all restraint protocols tested significantly alter blood pressure in this species of primates.

Introduction

Brazil has the highest diversity of primates in the world, with about 135 recognized taxa [11]. Among these, 70% are located in the Amazon region, making the area a true asset for biodiversity studies [7]. Feline night monkeys (*Aotus azarae infulatus*) are small neotropical primates with nocturnal habits. These monkeys are extensively used as animal models for biomedical research [13]. They are extremely susceptible to stress when handled, so the use of anesthetics and physical restraint methods should be carefully evaluated before any procedure [14].

The anesthetic techniques used in primates have an important role in veterinary medicine. In most cases, non-human primates can be agile or aggressive, so they require sedation or anesthesia to be submitted to clinical, diagnostic, or experimental procedures [22].

Several studies have been conducted to find the ideal anesthetics and tranquilizers that have low toxicity and that can be used in individuals of advanced age, with debilitation, or are easily stressed. The methods used should also have low cost and be easily acquired [23].

Blood pressure is an important parameter for the evaluation of the cardiovascular system [5]. Contradictions are found in the literature regarding the values of blood pressure (BP) considered normal among various animal species, which especially applies to primates. Additionally, there are few reports about BP in these animals. These values vary with age, sex, weight, and species, and there is no consensus about the most appropriate method of measurement for routine veterinary clinical examinations [18]. The objective of this study was to evaluate the influence of four different restraint protocols on blood pressure values measured in the neotropical non-human primates *Aotus azarae infulatus*

(feline night monkey), as both anesthetics and excessive agitation can alter the physiological values of these animals.

Materials and methods

We studied 10 adult male feline night monkeys, with ages ranged from five to eight years, which had an average weight of 1 kg and were clinically healthy. For identification and control, all animals had a three-letter code tattooed on the inner thigh and microchips implanted in the dorsal portion of the interscapular region. The subjects were captive born and belonged to the breeding colony of the National Primate Center (CENP) located in the Ananindeua municipality in state of Pará, Brazil (latitude 1°38′26″, longitude 48°38′22″). This project was approved by the Ethics in Research with Animals Committee UNESP—Botucatu (Protocol No. 215/2012-CEUA), and the Ministry of Environment—System Authorization and Information on Biodiversity—Sisbio (Protocol number 37034).

We evaluated the subjects' health condition via clinical exams (inspection, auscultation) and clinical laboratory testing, including hemogram and biochemical profile (alanine transaminase—ALT, aspartate transaminase—AST, alkaline phosphatase, albumin, total protein, urea, creatinine, and glucose). This was performed during the selection of the animals, which occurred 15 days before the start of proceedings. Only individuals considered healthy were selected for the experiment. Then, the 10 animals were subjected to four restraint protocols at one-week intervals to determine possible changes in BP values presented by the same organism when submitted to different restraint protocols. The procedures were randomized to avoiding interference on the results by acclimatizing, stress, or clinical status of the animals.

The same animals were used in the four stages of the project due to the fact that every individual has a different response to anesthesia. This study aims to evaluate the response of the same animal to the four restraint protocols, with the objective of verifying the changes introduced by the restraint protocols in BP values.

Capture and restraint methods

The animals were restrained manually using leather restraint gloves in their enclosure and placed in a plastic transport box. Then, they were transported to the exam room and remained in the box for 15 minutes before the procedure to reduce stress after handling. After that, the animal was taken out of the transport box and held by the animal keeper with the aid of leather restraint gloves. They were held manually in a supine position for the administration of anesthetic intramuscularly (Fig. 1A) or by mask (Fig. 1B), or they were held in the lateral decubitus position for examination.

The animals were weighed before any procedure to determine the exact amount of anesthetic to be administered. Weighing was carried out with the animals still inside the transport box based on the tare weight on an electronic scale, and then they were subjected to restraint protocols.

Restraint protocols

1 *ZOL*: In this group, tiletamine and zolazepam (Zoletil) were used at a dose of 5.5 mg/kg intramuscularly;
2 *ISO*: In this group, isoflurane anesthesia was provided through a facemask with oxygen;
3 *KET*: In this group, 15 mg/kg of ketamine and 1 mg/kg of midazolam were administered with the same syringe by the intramuscular route;
4 *PR*: In this group, the animals were restrained with leather restraint gloves and maintained in the lateral position for the exam. Additionally, condensed milk was administered to the animals with the aid of a tongue depressor to calm the animal for cooperation with the test, allowing the animal to ingest the liquid but without moving the arms or legs.

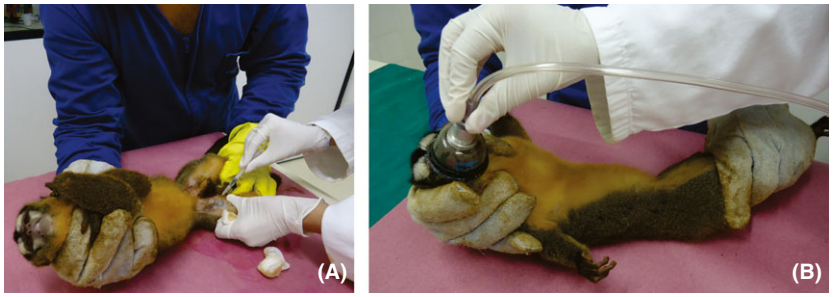

Fig. 1 Physical restraint in a supine position using leather restraint gloves for anesthetic administration by the intramuscular route **(A)** or through a mask **(B)**. Source: Research protocol.

J Med Primatol (2015) 1–6

After examination, the animals that had been subjected to the anesthetic protocols were kept in cages until the anesthesia passed and then moved to the enclosure.

Blood pressure determination

To perform the exam, the animal was positioned in the left lateral decubitus position to measure the BP of the right forearm (Fig. 2A) and then placed in the right lateral decubitus position to measure BP on the left forearm (Fig. 2B).

For BP measurement, we used Pet MAP (Ramsey Medical, Inc.; Florida-USA), which is a non-invasive tool for measuring blood pressure by the oscillometric method. This equipment has a set of seven cuffs of various sizes (2, 2.5, 3, 3.5, 4, 4.5, and 5.5 cm), which were chosen before the exam based on the animal size and circumference of the forearm (which was measured with the aid of a metric tape).

The cuffs were placed on the animal, and the exam only started after the animal was quiet. At the end of the procedure, the animal was placed in the transport box and brought to the enclosure, where it was released. To avoid the influence of different variables on the experiment, data were collected only after correct positioning of the animal on the table, because excitement from the physical restraint and improper anesthetic plane could influence the measured BP values.

Three measurements were performed on each forearm, with three pressure readings. Once positioned, the cuff was inflated and deflated automatically. The result of pressure was calculated automatically by a microprocessor inside the pressure machine and displayed on the pressure display panel in mmHg. Automatic measurements of systolic, diastolic, and mean blood pressure were obtained in three cycles with an interval of one minute for each forearm.

Statistical analysis

For comparison of the data obtained, we subjected the results to one-way ANOVA (variance analysis) with Tukey test to parametric data. The Wilcoxon test was used for comparison of data between arms, to verify whether there was significant difference between the results obtained from each arm. For statistical analysis, BioEstat 5.0 software was adopted [2]. All parametric tests performed satisfy the assumptions of normality and homoscedasticity. Statistical significance was determined by a p bilateral value equal to or less than 5% ($P \leq 0.05$).

Results

The distributions of heart rate (HR), systolic (SBP), diastolic (DBP), and mean blood pressure (MBP) are shown in Table 1. No statistically significant differences were found in BP values from each arm in all groups tested. There was no difference statistically significant between HR values in all protocols tested.

The ISO group presented statistical difference in SBP compared to ZOL ($P < 0.01$) and PR ($P < 0.01$) groups, but no significant difference was observed compared to the KET group. The analysis of SBP between the ZOL, KET, and PR groups showed no significant difference.

In DBP, ISO group showed statistically significant difference, with smaller values in relation to ZOL ($P < 0.05$), CET ($P < 0.01$) and CF ($P < 0.01$). It was also observed in PAM values, where ISO was statistically different from other groups evaluated (ZOL, $P < 0.05$; CET, $P < 0.01$; CF, $P < 0.01$).

Discussion

During the restraint procedures or the administration of anesthetics, clear signs of stress were observed in the form of defecation, urination, and escape attempts each time the animal was removed from the transport box. Monteiro et al. [15] reported that even animals born and raised in captivity are not adapted to restraint procedure.

Inhalation induction is the most common method of induction for pediatric patients without venous access [9] being considered very effective for induction in small

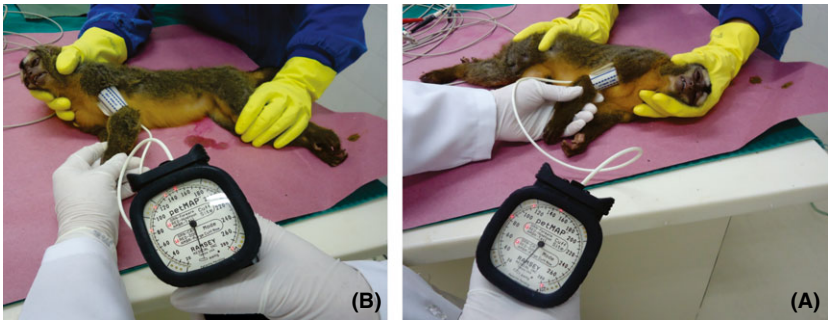

Fig. 2 Position of the animal to measure BP in the right arm **(A)** and left arm **(B)**. Source: Research protocol.

Table 1 Data obtained from the Pet Map blood pressure machine measured in *Aotus azarae infulatus* subjected to four restraint protocols

	Group							
	ZOL		ISO		KET		PR	
Parameters	RA	LA	RA	LA	RA	LA	RA	LA
HR	173.49 ± 47.49a*	180.55 ± 38.29a	174.99 ± 53.21a	192.87 ± 46.48a	165.49 ± 49.93a	164.08 ± 43.64a	190.83 ± 44.04a	171.66 ± 58.18a
Systolic BP	190.99 ± 31.82a	187.59 ± 36.25a	151.96 ± 23.89b	143.78 ± 21.93b	173.83 ± 20.63ab	172.57 ± 21.91ab	200.83 ± 24.14a	193.33 ± 29.40a
Diastolic BP	128.82 ± 21.28a	122.49 ± 22.29a	96.20 ± 21.10b	89.69 ± 23.62b	120.99 ± 17.67a	123.93 ± 28.18a	132.16 ± 19.08a	132.11 ± 21.93a
Mean BP	152.83 ± 24.62a	143.51 ± 26.29a	116.36 ± 20.05b	109.69 ± 22.51b	140.66 ± 16.23a	140.60 ± 21.72a	157.99 ± 18.01a	157.57 ± 22.79a

*The averages followed by the same letter horizontally do not differ by Tukey test ($P < 0.05$).

Source: Research protocol.

Data from CENP, Ananindeua, Pará, Brazil (2013).

HR = heart rate (bpm); BP = blood pressure (mmHg); RA = right arm; LA = left arm; ZOL=zoletil; ISO = isoflurane; KET = ketamine and midazolam; PR = physical restraint; (±) standard deviation.

primates, because only require a previous physical restraint [1]. This prevents stress caused by the administration of some injectable agent. Due to the small size of the animals, the mask induction was chosen because allow better control of anesthesia compared to the use of induction chamber.

HR was considered high in all protocols tested compared to data obtained by Smith and Astley [24] in *Aotus nancymai* in resting condition. However, the values found confirm what was described in other studies for anesthetized *Aotus azarae infulatus* [3, 8, 21]. It is known that all anesthetic protocols used increase HR, but stressing factors such as physical restraints also increase these values [24].

Ketamine produces more increases in HR, in contrast to midazolam, which usually does not alter this parameter [20]. Although tiletamine belongs to the same pharmacological group as ketamine, the association of tiletamine and zolazepam (Zoletil) produces minimal cardiovascular effects, whereas isoflurane may cause a slight decrease in HR [17]. Anesthesia can be a source of variability in the results of the BP, but the values of blood pressure found in this study are consistent with the references for *Aotus* under anesthesia [3, 21]. Smith and Astley [24] found similar values to those obtained in this work in *Aotus* subjected to a stressful stimulus, but they found significantly lower BP values in animals at rest that were not anesthetized. These results confirm the literature data about the pressure increase caused by ketamine [25] and Zoletil [10, 27].

Although midazolam is to be considered among the benzodiazepines, which further reduce blood pressure [12, 20], this was not observed in the KET group. However, pressure reduction occurred with the use of isoflurane, confirming literature reports [6, 16], and with findings by Vasconcellos et al. [26] in *Cebus apella*. In the physical restraint group, the blood pressure values were higher compared to the resting pressure [24]. This probably happened because of the stress caused by restraint, which causes the release of catecholamine, thereby increasing the values of BP. According to Smith and Astley [24], studies indicate that *Aotus* reacts to stressful situations in a laboratory environment, such as blood collection, with significant and prolonged elevations in blood pressure. Hypertension naturally occurs in *Aotus sp*. [24]. However, in this work, it was not possible to conclude whether the values found were the results of natural hypertension, the action of the anesthetics used, or the high level of stress caused by physical restraints.

Different measures of blood pressure in the arms may indicate a high risk for vascular disease [19]. This result reaffirms the importance of the guidelines established by

the Brazilian Society of Cardiology (for humans), in that it is necessary to measure blood pressure in both arms of the patient. In the present study, there was no significant statistical difference between the values obtained in the left arm and right arm in all restraint protocols tested. We chose to use an oscillometric method in determining the BP rather than the vascular Doppler method. Cabral et al. [4] concluded in a study on dogs that the results of SBP did not differ between the two methods, but the values found in DBP were not similar.

Conclusion

The present study on Feline night monkeys (*Aotus azarae infulatus*) indicates that all of the restraint protocols tested allowed for the determination of blood pressure with no difference in the values obtained in the right and left arms of the animals. Additionally, it was observed that all restraint protocols significantly raised blood pressure, however, animals in the isoflurane group showed lower values compared to the other groups. Methods that can verify the BP without restraint are very expensive and difficult to apply in some centers; therefore, it is suggested that research be expanded in this direction to define standards of normality in animals under restraint.

Acknowledgments

We are grateful to the National Primate Center (CENP) keepers and technicians for their support and commitment with this work.

References

1 Andrade A, Andrade MCR, Marinho AM, Ferreira FJ. Biologia, Manejo e Medicina de Primatas não humanos na pesquisa biomédica. Rio de Janeiro: FIOCRUZ, 2010. 472 p.
2 Ayres M, Ayres JM, Ayres DL, Santos AA. BIOESTAT – Aplicações estatísticas nas áreas das ciências bio-médicas. Belém, PA: Ong Mamiraua, 2007.
3 Baer JF: Husbandry and medical management of the owl monkey. In: Aotus: The owl monkey. Baer JF, Weller RE & Kakaoma I (eds). San Diego: Academic Press, 1994; 133–64.
4 Cabral RR, Ciasca BD, Oliveira VMC, Vaz-Curado AP, Larsson MHMA: Valores da pressão arterial em cães pelos métodos oscilométrico e Doppler vascular. *Arq Bras Med Vet Zootec* 2010; **62**:64–71.
5 Calçada D, Frazão J, Silva D. Pressão arterial. Lisboa. Monografia (Licenciatura em Engenharia Biomédica). Instituto Superior Técnico, Lisboa 2006, apud Silva, C.R.A. Métodos de aferição de pressão arterial em cães anestesiados com propofol e mantidos por halotano. Dissertação de Mestrado, Universidade Federal do Piauí, Teresina – Piauí 2010. Disponível em: http://www.ufpi.br/subsiteFiles/ciencianimal/arquivos/files/DM_CRAS.pdf
6 Clarke KW: Desflurane and sevoflurane: new volatile anesthetic agent. *Vet Clin North Am Small Anim Pract* 1999; **29**:793–808.
7 Del-Claro K, Fábio P. As distintas fases do comportamento animal. Sociedade Brasileira de Etologia, Jundiaí - SP: Livraria Conceito 2003: 276–7.
8 Galante R. Anestesia intravenosa total em primatas: comparação da infusão contínua de propofol com bolus intravenosos de tiletamina e zolazepam e associação de propofol com opioides ou cetamina. Dissertação de Mestrado. Universidade Federal do Paraná. Curitiba 2013. Disponível em: http://dspace.c3-sl.ufpr.br/dspace/bitstream/handle/1884/30418/R%20-%20D%20-%20RAFAELA%20GALANTE.pdf?sequence = 1.
9 Holzman RS, Mancuso TJ, Polaner DA: A Practical Approach to Pediatric Anesthesia. Philadelphia: Lippincott Williams & Wilkins, 2008; 688 p.
10 Hellyer P, Muir WW 3rd, Hubbell JA, Sally J: Cardiorespiratory effects of tiletamine/zolazepam to dogs. *Vet Surg* 1989; **18**:160–5.
11 ICMBio. Instituto Chico Mendes. Sumário executivo do plano de ação nacional para a conservação dos primatas do nordeste2011. Disponível em: http://www.icmbio.-gov.br/portal/images/stories/docs-plano-de-acao/pan-primatas-caatinga/sumario-primatas-nordeste-web.pdf
12 Lebowitz PW, Cote ME, Daniels AL, Martyn JA, Teplick RS, Davison JK, Sunder N: Cardiovascular effects of midazolam and thiopentone for induction of anesthesia in ill surgical patients. *Can J Anaesth* 1983; **30**:19–23.
13 Menezes AN, Bonvicino CR, Seuanez HN. Identification, classification and evolution of owl monkeys. Aotus, Illiger 1811. *BMC Evol Biol* 2010; **10**: 248.
14 Monteiro FOB. Avaliação uterina e diagnóstico de gestação em macaco-da-noite (*Aotus azarae infulatus*) por ultra-som em modo B. Tese de Mestrado. Jaboticabal, SP 2004. Disponível em: http://base.repositorio.unesp.br/bitstream/handle/11449/98208/mon-

teiro_fob_me_jabo.pdf?sequence = 1&isAllowed = y.
15 Monteiro FOB, Koivisto MB, Vicente WR, Amorim CR, Whiteman CW, Castro PH, Maia CE: Uterine evaluation and gestation diagnosis in owl monkey (*Aotus azarai infulatus*) using the B mode ultrasound. *J Med Primatol* 2006; **35**:123–30.
16 Mutoh T, Nishimura R, Kim H, Matsunaga S, Sasaki N: Cardiopulmonary effects of sevoflurane, compared with halothane, enflurane, and isoflurane, in dogs. *Am J Vet Res* 1997; **58**:885–90.
17 Natalini CC. Teorias e técnicas em anestesiologia veterinária. Porto Alegre: ArtMed, 2007: 53–6.
18 Nelson RW, Couto CG. Medicina interna de pequenos animais. 4 edn. Rio de Janeiro: Elsevier, 2010; cap 11: 184–91.
19 News.Med.Br. Diferença entre pressão arterial sistólica medida nos dois braços pode indicar doenças vasculares e risco de mortalidade, de acordo com publicação do The Lancet**.** Medical Journal 2012. Disponível em: http://www.news.-med.br/p/medical-journal/258605/diferenca-entre-pressao-arterial-sistolica-medida-nos-dois-bracos-pode-indicar-doencas-vasculares-e-risco-de-mortalidade-de-acordo-com-publicacao-do-the-lancet.htm. Acesso em: 7 July 2013.
20 Ozturk T, Tuncok Y, Kalkan S, Guven H, Aran G: Midazolams cardiac depressant effects and their lack of reversal by flumazenil in isolated rabbit hearts. *Pharmacol Res* 1999; **39**:283–7.
21 Rajendra RS, Brady AG, Parks VL, Massey CV, Gibson SV, Abee CR: The normal and abnormal owl monkey (*Aotus sp*.) heart: looking at cardiomyopathy changes with echocardiography and electrocardiography. *J Med Primatol* 2010; **39**:143–50.
22 Sainsbury AW, Eaton BD, Cooper JE: Restraint and anaesthesia of primates. *Vet Rec* 1989; **125**:640–3.
23 Silva ALP, Ferreira HI: O uso do cloridrato de detomidina em cães. *Cães Gatos* 1994; **9**:18–20.
24 Smith OA, Astley CA: Naturally occurring hypertension New World nonhuman primates: potential role of the perifornical hypothalamus. *Am J Physiol* 2006; **292**:937–45.
25 Souza KM, Vinagre RCO. Cetamina - Anestesia Venosa. SAERJ - Sociedade de Anestesiologia do Estado do Rio de Janeiro 2004. Disponível em: http://www.saerj.org.br/download/livro%202004/07_2004.pdf
26 Vasconcellos CHC, Mársico Filho F, Gomez Segura IA, Nascimento PRL, Monteiro RV. Utilização do isoflurano em macacos-prego (*Cebus apella* - Cebidae, Primata). *Braz J Vet Res Anim Sci*2000; **37**: 65–9.
27 Wilson RP, Zagon IS, Larach DR, Lang CM: Cardiovascular and respiratory effects of tiletamine-zolazepam. *Pharmacol Biochem Behav* 1993; **44**:1–8.