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Anatomy of the basal nuclei of *Alouatta belzebul*

Anatomia dos núcleos da base de Alouatta belzebul

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Abstract
The basal nuclei are well-defined bodies of neurons with specific functions, located inside the white medullary center of the brain, directly involved with the motor system, participating greatly in the planning and control processes of movements. Studies on these nuclei in non-human primates are small and in the *Alouatta belzebul* species, nonexistent. The aim of the present study was to describe the morphology of the nuclei at the base of the brain of *Alouatta belzebul*. Ten male and female *Alouatta belzebul* brains were used, where after removal and coronal cut of the brain, the Mayland technique was performed to show the basal nuclei. There was the presence of the caudate nucleus, lentiform nucleus (this formed by the putamen, medial globus pallidus and lateral globus pallidus), claustrum and substantia nigra, which, functionally, are related to motor control. The substantia nigra is part of the midbrain and is also related to learning resulting from the effects of dopamine, responsible for activating the reward and addiction system in the telbrain and is also related to the red nucleus, which is also a midbrain nucleus. In *Alouatta belzebul* the red nucleus is present. It was found in the literature that degeneration of substantia nigra cells can cause Parkinson's disease in *Macaca fasciculares*, and because *Alouatta belzebul* has the same anatomical structures in the basal nuclei of the base of *Macaca fasciculares*, it is suggested that studies of functional evaluation of these structures should be carried out to verify whether *Alouatta belzebul* can be used as an experimental model for Parkinson's disease.
Keywords: Basal ganglia; Substantia nigra; Putamen; Globe pale; Howler monkey.

Resumo
Os núcleos da base são corpos de neurônios, bem delimitados e com funções específicas, localizados no interior do centro medular branco do cérebro, envolvidos diretamente com o sistema motor, através de uma função moduladora dos movimentos, participando sobremaneira nos processos de planejamento e controle dos movimentos. Os estudos sobre estes núcleos em primatas são reduzidos e na espécie *Alouatta belzebul*, inexistente. O objetivo do presente estudo foi descrever a morfologia dos núcleos da base do encéfalo de *Alouatta belzebul.* Para tanto, foram utilizados dez encéfalos de *Alouatta belzebul*, machos e fêmeas, onde após a remoção e corte coronal do cérebro, realizou-se à técnica de Mayland para evidenciar os núcleos da base. Verificou-se a presença do núcleo caudado, núcleo lentiforme (este formado pelo putâmen, globo pálido medial e globo pálido lateral), claustro e substância negra, que, funcionalmente, estão relacionados com o controle motor. A substância negra faz parte do mesencéfalo e está ainda relacionada com a aprendizagem decorrentes dos efeitos da dopamina, responsável por ativar o sistema de recompensa e vício no telencéfalo e tem ainda, relação com o núcleo rubro que também é um núcleo do mesencéfalo. Em *Alouatta belzebul* o núcleo rubro está presente. Verificou-se na literatura que a degeneração de células da substância negra pode ocasionar a doença de Parkinson em *Macaca fasciculares*, e pelo fato do *Alouatta belzebul* apresentar as mesmas estruturas anatômicas dos núcleos da base do mesencéfalo de *Macaca fasciculares*, poderia ser utilizado como modelo experimental em estudos clínicos para a doença de Parkinson.
Palavras-chave: Núcleo da base; Substância negra; Putâmen; Globo pálido; bugio.

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Introduction

The *Alouatta belzebul* species is endemic from Brazil and happens in a variety of habitats, including the floodplain amazon forest, the floodplain forest of Marajó and fragments of the northern Atlantic Forest [(1)]. According to the Red List of Endangered Brazilian Fauna classification, updated in 2018, the specie *Alouatta belzebul* categorized as vulnerable. A species is vulnerable when the available evidences indicate that it faces a high risk of extinction in nature, in the very near future. In the case of *Alouatta belzebul*, the classification as vulnerable is due to the fact that the species has decreased by at least 30% in the last 36 years (three generations). In the Brazilian Atlantic Forest, the isolated

subpopulation is in a more critical condition, with only 200 individuals surviving in isolated fragments in the northeast coast [2,3].

The descriptive study of the anatomy of wild animals' experiences, nowadays, undeniable importance. The great proximity of non-human primates to man allows adopting them as a model for human research in several areas such as: anatomy, physiology, endocrinology, immunology and biotechnology in reproduction [1]. Improved knowledge of the anatomy and, in particular, of the nervous system can represent an important factor for the preservation, protection and understanding of the evolution of Neotropical primates [4-8].

Macroscopic anatomy serves as a tool of fundamental importance for the description of a species and/or for the comparison between species that present morphological similarities and the mesoscopic and microscopic anatomy complements it, since it addresses the organization and function of tissues. The morphological information about the base nuclei allows the understanding of motor behavior and ethological and behavioral aspects of different species, as well as supports the development of conservation strategies [7,9,10]. The species *Alouatta belzebul* was evaluated in other anatomical studies, such as the spinal cord [11], vascularization of the brain [12], digestive tract [13], dura mater venous sinuses [14] and female reproductive system [15], however, the islands of grey substance (cellular bodies) inside the white substance of the brain and of extreme importance for motor control called basal nuclei, were not studied in this species.

The basal nuclei are composed of masses of gray substance (nuclei) located inside the medullary white center of the brain which is the white substance of the telencephalon. Originally, only five nuclei were considered from a strictly anatomical point of view: the caudate nucleus, the putamen, the globus pallidus, the amyloid body and the claustrum [16]. Of these structures, only two are not directly related to motor function: the amyloid body (related to emotional behavior and memory in humans) and the claustrum (of unknown function, although it is speculated to be related to visual function). The putamen and the globus pallidus constitute the lentiform nucleus and this, together with the caudate nucleus forms the so-called striated body [17].

In the broader functional sense, the scientific literature also considers as part of the basal nuclei certain structures that maintain important connections and intimate functional relationships with the "true" basal nuclei, namely: the substantia nigra (situated in the midbrain), the subtalamic nucleus (the main constituent of the subtalamo in the diencephalon) and the pontinal peduncular tegmental nucleus (situated in the pontinal parabrachial reticular formation) [18,19]. This study aimed to identify and describe the mesoscopic anatomy of the nuclei of the *Alouatta belzebul*.

Materials and methods

For this study ten *Alouatta belzebul* encephalus, males and females, adults and free of injuries, collected during the period of rescue and rescue of land fauna during the implementation activities of the Belo Monte Hydroelectric Plant - Brasília-DF, governed by the process of IBAMA - Brazilian Institute of Environment and Natural Resources, n°. 02001.001848/2006-75 and authorization No. 473/2014 were used. After the rescue, the animals were frozen and sent to the Human and Comparative Anatomy Laboratory of the Federal University of Jataí, being kept frozen until the beginning of the processing. The experimental procedure was approved by the Ethics Committee on the Use of Animals of the Federal University of Goiás (UFG) (protocol number 083/17).

All animals were weighed and then kept in 10% formaldehyde aqueous solution by intramuscular, subcutaneous and intracavitary injections. The specimens were kept in this solution for at least 72 hours. After the fixation period, the brains were carefully removed from the skull, removing the cranial cap with the aid of an oscillating saw (Dremel® 3000) in the cranium-caudal direction, from the height of the frontal bone to the occipital, in order to maintain encephalic integrity. The brain cells after removal were weighed in a digital analytical balance (model EEQ9003F-B Edutec® brand) and measured with an MTX® brand digital pachymeter and documented with a digital camera, they were kept in 10% formaldehyde solution.

For the mesoscopic analysis of the base nuclei, the Mayland technique was performed. As a preparation, serial frontal cuts of 10 mm thickness of the brain of *Alouatta belzebul* were carried out, resting the material on a flat surface, with the help of 10 mm markers and sharp-cut blade. 15 sections were separated from each telencephalon, placed in oxygenated water at 10 volumes for 24 hours for whitening and then washed in running water. The technique described by Mainland [20] and Merini et al. [21] was performed as follows: the cuts were submerged for 5 minutes in a 1% ferric chloride solution, then washed in running water. After this step, the cuts were immersed in a 1% ferrocyanide solution until the desired coloration was obtained (normally for about 30 seconds), and washed again in running water. After coloring, the pieces were placed in 1% nitric acid solution for 24 hours. For conservation, the pieces remained in a 5% formaldehyde solution. For nomenclature the Veterinary Anatomic Nomina (2017) [22] and International Anatomical Terminology [23] were used.

Results and discussions

The base nuclei observed in *Alouatta belzebul* as a set of subcortical nuclei located in the basilar portion of the cerebral hemispheres were identified as caudate nucleus, lentiform nucleus (this one formed by the putamen, medial globus pallidus and lateral globus pallidus), claustrum and substantia nigra (Figure 1 and 2).

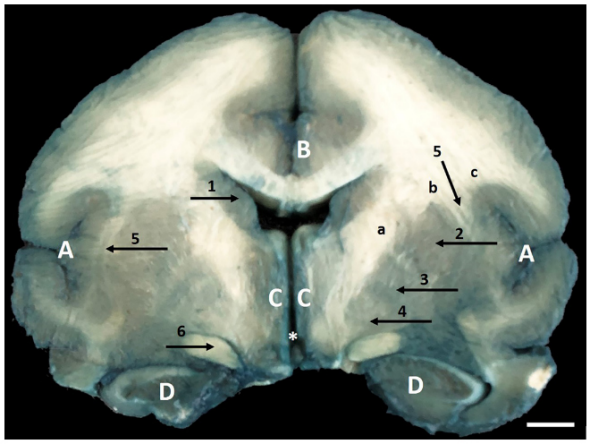

Figure 1. View in coronal cut of *Alouatta belzebul*'s brain (A) Insula wolf(B) Cingulate gyrus; (C) Thalamus; (D) Hippocampus; (*) Third ventricle; (1) Caudate nucleus (body); (2) Putamen; (3) Lateral globus pallidus; (4) Medial Globus Pallidus; (5) Claustrum; (6) Optical tract; (a) Internal capsule (posterior branch); (b) External capsule; (c) Extreme capsule. (Bar 0.5 cm).

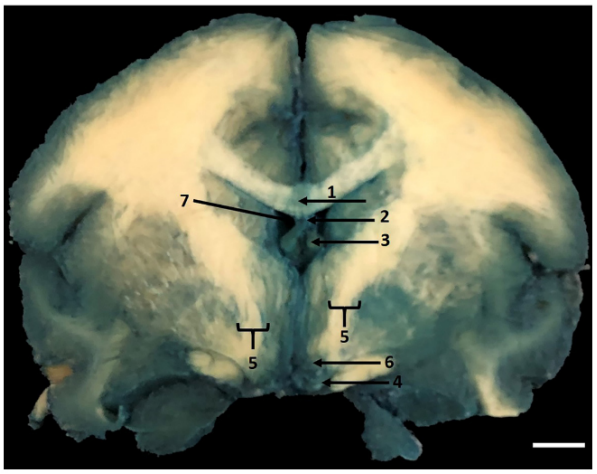

Figure 2. View in coronal cut of the *Alouatta belzebul* brain. (1) Corpus callosum (trunk), (2) Septum pellucidus; (3) Fornix (branch); (4) Substantia nigra; (5) Peduncle cerebral; (6) red nucleus and (7) lateral ventricle. (Bar 0.5cm).

The *Alouatta belzebul* caudate nucleus was composed of a gray substance, located near the lateral wall of the lateral ventricles. This nucleus is composed of head, body and tail, where the head corresponds to its anterior portion protruding into the anterior horn of the lateral ventricle, being laterally delimited by the putamen and the anterior arm of the inner capsule. These findings corroborate with data from Watanabe and Madeira [24] and Santos [25] in *Sapajus libidinosus*, Geist [26] in Rhesus and by Machado and Haertel [17] in *Homo*.

The lentiform nucleus is separated medially from the caudate nucleus and thalamus by the inner capsule and laterally from the claustrum by the outer capsule. In the globus pallidus in *Alouatta belzebul* two portions were identified, a pale medial globe and a pale lateral globe in both cerebral hemispheres, separated by the internal medullar slide. This blade is difficult to visualize in this species. Santos [25] describes that at the time of the optical chiasm in *Sapajus libidinosus*, the globus pallidus was observed well demarcated, but without its divisions. However, in more caudal cuts it was possible to see the division of two segments, separated by the medullar blade, as well as in *Alouatta belzebul*. The putamen was the biggest observed nucleus and it is constituted of gray substance, which contains neuron bodies.

The caudate and lentiform nuclei together make up the striated body, which functionally represents the most important center of extrapyramidal somatic motricity in *Homo* [18,19].

In the striated body of human and non-human primates, it is possible to identify a division into neoestriate and palaeestriate. The neoestriate is the most recent phylogenetically portion and comprises the caudate and putamen nucleus, that is, the lateral portion of the lentiform nucleus and the paleoestriate is the oldest phylogenetically portion and concerns the globus pallidus, that is, the medial portion of the lentiform nucleus [16]. All of these structures were onserved in *Alouatta belzebul.*

The claustrum in *Alouatta belzebul* is a thin and extensive strip of grey substance where the neurons are connected to the cerebral cortex, situated between the extreme capsule (which separates from the insular cortex) and the external capsule (which separates the putamen), two formations of white substance (Figure 2). In this species, the external capsule together with the claustrum and the extreme capsule extend from the region of the medullar white center, located in the dorsal part of the brain, and are at its ventral end with the medullar white center in the region of the temporal lobes, as was also observed and described by Machado and Haertel [17] in *Homo*, Watanabe and Madeira [24] and Santos [25] in *Sapajus libidinosus*, and Geist [26] in *Macaca mulatta*. Studies in lower mammals [27] revealed that the cerebral cortex sends projections to the claustrum, with an intense bilateral claustrocortical projection, although with ipsilateral prevalence. The claustrum is also part of the limbic system, with dorsolateral projections to the insula lobe. This projection

was not found in *Callithrix* [28].

In the medial face of the brain and midbrain other anatomical structures were identified in *Alouatta belzebul*: thalamus, corpus callosum, pellucid septum, fornix (branch), substantia nigra, cerebral peduncle, red nucleus and lateral ventricle.

The substantia nigra is a subcortical nucleus traditionally related to the base nuclei. It is composed in *Alouatta belzebul* by melanin pigmented neurons, which give the substantia nigra its dark coloration and is interconnected with the striated body structures (Figure 2). Tilney [29] identified in *Tarsius* a bigger and diffuse substantia nigra when compared to other primates, as species of the genera *Pan*, *Papio* and *Macaca*. The fact that it has a small telencephalon and a large, diffuse substantia nigra is related to its low motor performance. According to Noureldine [19], in *Homo*, the substantia nigra is associated with learning processes because it mediates the brain response to certain stimuli. Its function is to facilitate learning, thanks to the reinforcing effects of dopamine, and in addition, it engages in spatial learning. Dopamine is responsible for activating the reward system and addiction in the telencephalon, and is related to the presence of the red nucleus. In *Alouatta belzebul* the red nucleus is present (Figure 2). The subtalamic nucleus was not observed in *Alouatta belzebul.* No difference was observed in the anatomy of the basal nuclei between males and females.

The lack or reduction of dopamine affects the movements of the body resulting from the degeneration of cells of the substantia nigra. This reduction may cause Parkinson's disease in non-human primates, according to a study published by Kikuchi et al. [30] that in *Macaca fascicular* verified techniques of Parkinson's reversion through the introduction of pluripotent cells, induced to repair the function of the substantia nigra and red nucleus, achieving positive results for this primate to obtain the recovery of several movements. Thus, we believe that because *Alouatta belzebul* presents these same morphological structures as *Macaca fascicular*, it is suggested that studies of functional evaluation of these structures should be carried out to verify whether *Alouatta belzebul* can be used as an experimental model for Parkinson's disease.

Conclusion

In the analysis of the base nuclei, little studied in non-human primates, the presence of the caudate nucleus, putamen, medial globus pallidus and lateral globus pallidus, claustrum and substantia nigra, which are functionally related to motor control, was quite evident. The substantia nigra is also related to learning from the effects of dopamine, responsible for activating the system of reward and addiction in the telencephalon and also has a relationship with the red core. In *Alouatta belzebul* the red nucleus is present. It was found in the literature that degeneration of substantia nigra cells can cause Parkinson's disease in *Macaca fasciculares*, and because *Alouatta belzebul* has the same anatomical structures in the basal nuclei of the base of *Macaca fasciculares*, it is suggested that studies of functional evaluation of these structures should be carried out to verify whether *Alouatta belzebul* can be used as an experimental model for Parkinson's disease.

Conflict of interest statement

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.K.S. Pereira, F.R. de Melo, F. C. S. A. de Melo, K. F. Pereira, V. A. S. Vulcani; Data curation: D.K.S. Pereira; Project administration: D.K.S. Pereira; Resources: F.R. de Melo; Supervision: V. A. S. Vulcani; Writing – original draft: D.K.S. Pereira, K. F. Pereira, V. A. S. Vulcani; Writing – review & editing: D.K.S. Pereira, K. F. Pereira, V. A. S. Vulcani.

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References

1. Nascimento FF, Bonvicino CR, de Oliveira MM, Schneider MPC, Seuánez HN. Population Genetic Studies of *Alouatta belzebul* from the Amazonian and Atlantic Forests. American Journal of Primatology. 2008; 70: 423-431.

2. Valença-Montenegro, M.M., Fialho, M.S., Carvalho, A.S., Ravetta, A.L., Régis, T., de Melo, F.R., Jerusalinsky, L., Veiga, L.M., Mittermeier, R.A., Cortes-Ortíz, L. & Talebi, M. 2021. Alouatta belzebul (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2021: e.T39957A190412426. https://dx.doi.org/10.2305/IUCN.UK.2021-1.RLTS.T39957A190412426.en. Downloaded on 26 September 2021.

3. ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume II–Mamíferos. Availeble on: http://icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/livro_vermelho_2018_vol2.pdf.

4. Amado LTM, Sousa GC, Silva DCO, Silva Z, Júnior RB, Neto MAF, Lizardo FB, Santos LA, Barros RAC, Santos ALQ. Anatomia da fixação proximal do músculo reto femoral em humanos, *Cebus apella* e *Alouatta guariba*. Pubvet. 2011; 5: 1072-1078.

5. Leonel LCPC, Lima TC, Felipe RL, Silva EM, Silva GAO, Silva DCO, Carvalho-Barros RA, Silva Z. Anatomia descritiva da traqueia do macaco-prego (*Sapajus apella*). Biotemas. 2013; 26: 179-183.

6. Mayor P, Bowler M, Lopez APC. Functional morphology of the female genital organs in the peruvian red uakari monkey (*Cacajao calvus ucayalii*). Am. J. Primatol. 2013; 75: 545–554.

7. Lopes GP, Brito AB, Paim FP, Santos RR. Comparative characterization of the external genitalia and reproductive tubular organs of three species of the genus *Saimiri Voigt*, 1831 (Primates: Cebidae). Anatomia, Histologia Embryologia. 2016; 46: 143-161.

8. Silva EV, Silva SF, Aversi-Ferreira RAGMF, Abreu T. Nishijo H, Aversi-Ferreira TA. Comparative anatomy of the pelvic nerves in bearded capuchins (*Sapajus sp*). Braz. J. Vet. Res. Anim. Sci. 2016; 53: 1-17.

9. Teixeira DG, Hamlett WC, Guimarães MABV, Morini AC, Araújo KPC, Cury FS, Souza AF, Vidane AS, Ambrósio CE, Miglino MA. Morphological Tools for Describing the Male External Genitalia of *Sapajus apella*. Zoolog. Sci. 2015; 32: 97-104.

10.Lima AR, Guimarães SB, Branco E, Giese, EG, Muniz JAPC, Ricci REG, Miglino MA. Anatomy and histology of the urinary tract in the capuchin monkey (*Sapajus apela*). Pesq. Vet. Bras. 2016; 36: 221-226.

11.Souza-Terra DR, Sabec-Pereira DK, Lima FC, Melo FCSA, Melo FR, Pereira KF: Anatomy of the spinal cord of *Alouatta belzebul*. Acta Veterinaria Brasilica. 2018; 12: 55-61.

12.Sabec-Pereira DK, Lima FC, Melo FR, Melo FCSA, Pereira KF, Vulcani VAS: Vascularization of the *Alouatta belzebul* brain base. Pesq. Vet. Bras. 2020; 40: 315-323.

13.Segantine ACL, Melo FCSA, Melo FR, Schell RK, Zarpelon-Schutz AC, Sabec-Pereira DK, Pereira KF. Morfologia do tubo digestório de *Alouatta belzebul*. Research, Society and Development, 2020; 9: e5229108930.

14.Sabec-Pereira DK, Melo FR, Melo FCSA, Pereira KF, Vulcani VAS: Anatomy of the dura mater venous sinus of *Alouatta belzebul*. Anat Histol Embryol, 2020; 50: 58-64.

15.Pereira ER, Pires VCMC, Fernandes RJ, Sabec-Pereira DK, Melo FR, Schell RKW, Zarpelon-Schutz AC, Pereira KF. Anatomia do sistema reprodutor feminino de *Alouatta belzebul* (Linnaeus, 1766). Arq. Bras. Med. Vet. Zootec. 2020; 72: 2101-2110.

16.Prada I. Neuroanatomia funcional em medicina veterinária com correlações clínicas. Jaboticabal: Terra Molhada; 2014.

17.Machado ABM, Haertel LM. Neuroanatomia Funcional. São Paulo: Atheneu; 2014.

18.Meneses MS. Neuroanatomia aplicada. São Paulo: Grupo Gen-Guanabara Koogan; 2016.

19.Noureldine MHA. Fundamentos da neuroanatomia: um guia clínico. Rio de Janeiro: Elsevier; 2019.

20.Mainland D. Uber makroskopiche faerburg von Gehirnprraeparaten mit Berlinblau. Anat Anz. 1926; 65: 85-88.

21.Merini TT, Krum LK, Colman J. *et al*. Comparative analysis of human brain with two diferent staining techniques. UEPG Ci. Biol. Saúde. 2014; 20: 99-104.

22.Word Association of Veterinary Anatomists:. Nomina Anatomica Veterinaria. Ithaca: International Comittee On Veterinary Gross Anatomical Nomenclature. 2017; 35-160.

23.Sociedade Brasileira de Anatomia. International Anatomical Terminology. Anatomical CdT. São Paulo: Editora Manole; 2001.

24.Watanabe L, Madeira MC. The anatomy of the brain of the tufted capuchin (*Cebus apella* LINNAEUS, 1758). Rev. Odont. UNESP. 1982; 11: 5–12.

25.Santos JML. Estruturação de uma plataforma de ensino referente à constituição anatômica do Sistema Nervoso Central do Macaco-prego (*Cebus apella*). São Paulo: USP. 2019; 25-85.

26.Geist FD. The brain of the Rhesus monkey. Journal of Comparative Neurology, 1930; 50: 333-375.

27.Machado ABM. Neuroanatomia Funcional. São Paulo: Atheneu, 1993, 140-224.

28.Reser DH, Richardson KE, Montibeller MO, Zao S, Chan JMH, Soares JGM, Chaplin TA, Gattass R, Rosal MGP. Claustrum projections to prefrontal cortex in the capuchin monkey (*Cebus apella*). Frontiers in systems neuroscience, 2014; 8: 1–10.

29.Tilney F. The brain stem of tarsius. A critical comparison with other primates. The Journal of Comparative Neurology, 1927; 43: 371-432.

30.Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, Mizuma H, Takara S, Takahashi R, Inoue H, Morita S, Yamamoto M, Okita K, Nakagawa M, Parmar M, Takahashi J. Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature. 201; 548: 592–596.