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ARTERIAL CIRCLE OF THE AFRICAN GREEN MONKEY BRAIN

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This research has been carried out on 20 African green monkey brains (Cercopithecus aethiops) whose cerebral vascular bed has been injected with Latex. After fixation in 10% formalin solution, arteries of the brain base have been put through stereomicroscopic dissection and measurements. It was determined that on the brain base of this monkey there is an arterial circle (AC), which is formed, anteriorly, by the terminal parts of both internal carotid arteries and their branches, i.e. the right and left anterior cerebral arteries, and posteriorly, by terminal branches of basilar artery, i.e. the right and left posterior cerebral arteries, as well as right and left posterior communicating arteries. The length and diameter of the arteries that constitute the AC of the Cercopithecus brain base have been measured. On the basis of the measured values, the length and the diameter of the anterior (left, right, total) and posterior segment (left, right, total) of the AC have been calculated. The measurements point towards mild L/R asymmetry of the AC segments, as well as towards differences between their anterior and posterior segments. However, the pointed L/R asymmetry is not significant ($p>0.05$) for the values for length ($F_B=0.033$; $p=0.856$), nor for the values of diameters ($F_B=1.344$; $p=0.250$). The anterior and posterior segments of the AC do not differ significantly ($p>0.05$) according to the length ($F_A=102.194$; $p=0.962$; $t=-1.103$; $p=0.277$). Diameters of the anterior segment have a highly significant difference ($p<0.01$) compared to the diameters of the posterior segment of the brain base AC ($F_A=102.194$; $p=0.00$; $F_{dL}=58.139$; $F_{dD}=43.000$; $t=7.651$; $p=0.00$). The African green monkey with the described similarities of the brain base arterial circle, which correlates to that of the humans, may represent a suitable experimental animal model in complex cardiovascular research.

Key words: African green monkey, Cercopithecus, arterial circle of the brain, anatomy, morphometry

INTRODUCTION

In the developed countries of the Western world cardiovascular diseases take the greatest toll in deaths. Only in the USA, 7.600.000 people per year suffer a myocardial infarction (Adams and Apple, 2004). Coronary arterial disease which precedes all others cannot be viewed separately. It has been shown that the degree of atherosclerotic changes of the coronary arteries, correlates with the changes in the carotids. Recently it has been reported that the abnormalities of the caliber of the internal carotid arteries have been conjoined with the anomalies of the circle of Willis (Macchi *et al*., 2003). *Circulus arteriosus* cerebri (Willis) has been a subject of many studies, since the anomalies located on it were more of a rule than an exception (Rothberg *et al*., 1977), thus they have presented a challenge for numerous researchers (Batujeff, 1889; Peli, 1902; Adachi, 1928; Mitterwallner, 1955; Servida and Mortillaro, 1962; Cavallotti *et al*., 1980; Yamada *et al*., 1995; Kawaguchi *et al.*, 1996; Hartkamp *et al*., 1999; Malobabić *et al*., 2000; Hedrikse *et al*., 2001; Kapoor *et al*., 2003). However, numerical data related to the arteries that form the circle of Willis are not so common (Orlandini, 1970; Gulisano *et al*., 1982; Orlandini *et al*., 1985). On the other side, when research has shown that monkey hearts were more suitable as an experimental model than the dog hearts (Buss *et al*., 1982; Teofilovski *et al*., 1988; Teofilovski *et al.*, 1992; Teofilovski-Parapid and Kreclović, 1998) we became interested in the arterial vascularisation of the brain in the African green monkey, whose coronary scheme we had a chance to study. Certain parts of the African green monkeys brain show similarities to those of the humans (Teofilovski *et al*., 1983, 1986; Teofilovski, 1984; Teofilovski-Parapid *et al*., 1991).

In this research we were primarily concerned with the source of the cerebral circulation of the African green monkey, i.e. the arterial circle on the brain base. The goal of this research was to establish, describe and analyse the length and diameter of the anterior and posterior segment of the arterial circle of the brain base of the African green monkey.

MATERIAL AND METHODS

The research encompassed 20 African green monkeys (*Cercopithecus aethiops*) (GA-monkey), imported from Kenya. The animals were healthy, and were sacrificed after a six week quarantine in which they were placed upon import, and have not shown any signs of disease by the end of it. Their body mass varied between 2000 and 3800 gr., and the age determined on the base of dentition was from 4 to 5 years.

The animals were kept in pairs in cages made of steel wiring (1m x 1m x 1m) in a room where the temperature was maintained at 25±2^{o}C. At the time of the experiment artificial lighting was not used. They were fed daily with a special commercial feed (SDS-ESSEX, UK) with the addition of bananas and apples.

The applied method in this research consisted of the following steps: a) to prepare the specimens (sacrificing the animals, injecting the blood vessels, fixation of tissues and dissection); b) to identify the arterial circle (locating the

adequate arteries on the brain base); c) to measure the lengths and external diameters of the arteries which make up the arterial circle (AC) of the brain base; d) to calculate the lengths and external diameters of the anterior (left, right, total) and posterior (left, right, total) segments of the arterial circle of the brain base; e) to explain the length and diameters of the anterior (left, right, total) and posterior (left, right, total) segments of the arterial circle by calculating the measures of central tendency and measures of variability; f) to analyse the differences in lengths and diameters of anterior (left, right, total) and posterior (left, right, total) segments of the arterial circle of the brain base.

The animals were anesthetized using Ketamin (RALATEK – Hemofarm, SMG) in a 15 mg/kg dose and then were sacrificed by bleeding-out. The brain vascular bed was perfused *in situ* with a 10% formalin solution, and then colored Latex was injected through the thoracic aorta. Colored Latex was used to secure a better visualization of the delicate blood vessels. Latex injection was not possible through the carotids due to their extremely small dimensions. After injection, the heads were sunk with the torso in a 10% formalin solution. After a three week fixation period, craniotomies were performed and the brains were removed from the skull.

Arteries of the brain base were microdissected with minimal damage of the surrounding brain tissue. The preparations were then photographed, so that they could finally be put through stereomicroscopic measuring. The external diameters of the posterior cerebral arteries (at the beginning of the artery) were measured, both of the internal carotid arteries (the end segments), anterior cerebral arteries (just before the joining), and posterior communicating arteries (in its mid segment). We also measured the length of the appropriate segments of the arteries which form the arterial circle of the brain base.

In this research the following statistical methods were used: measure of central tendency (arithmetical mean – $\bar{x}$), measures of variability (interval variations – I, standard deviation – sd, coefficient of variation – cv), Student's T test (t), parametric bifactorial variant analysis (F_A, F_B, F_{AB}) and the Fischer method of the least significant difference – LSD (F_d).

RESULTS

Vascularisation of the GA-monkey's brain comes from two great paired arterial vessels, the right and left internal carotid artery (*a.carotis interna dextra et sinistra*) and the right and left vertebral arteries (*a. vertebralis dextra et sinistra*). Both vertebral arteries are joined in the skull into a single vessel, the basilar artery (*a.basilaris*), which, coming to the prepontine groove separates into two terminal branches, the right and left posterior cerebral artery (*a.cerebri posterior dextra et sinistra*). Finally, on the brain base, the terminal end of the internal carotid artery and its branch, anterior cerebral artery (*a.cerebri anterior*), join with the help of the posterior communicanting artery (*a.communicans posterior*) with the posterior cerebral artery of the corresponding side (Figure 1). So, on the base of the brain, an arterial circle is formed, similar to that of the humans' circle of Willis. Different to the human brain, on its anterior part, occurred a joining of both anterior cerebral

arteries (*a.cerebri anterior dextra et sinistra*) into a single vessel, the anterior common cerebral artery (*a.cerebri anterior communis*). Therefore, anterior segment of the arterial circle of the GA-monkey brain is formed by the terminal parts of both of the internal carotid arteries and both anterior cerebral arteries, and the posterior segment of both of the posterior communicating arteries and both posterior cerebral arteries.

In a certain number of animals, L/R discrepancies in the appearances of the arterial circle of the GA-monkeys' brains were noticed. In our study we had one brain with a hypoplastic right anterior cerebral artery (Figure 2), one with a fenestration of the same vessel (Figure 1) and one with a hypoplastic right posterior communicating artery.

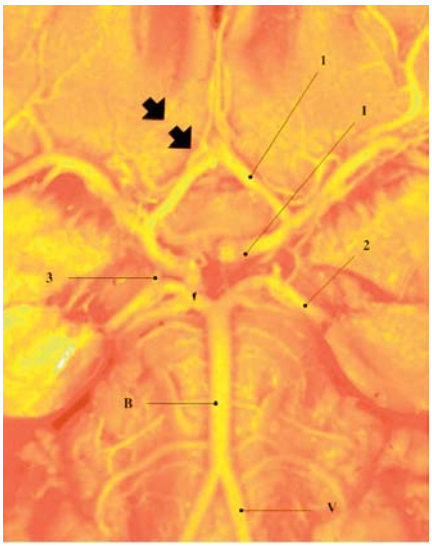

Figure 1. Arterial circle of the *Cercopithecus aethiops* brain base: Internal carotid artery (I), anterior cerebral artery (1), posterior cerebral artery (2), posterior communicating artery (3). Fenestration of the right anterior cerebral artery (arrow). Basilar (B) and vertebral (V) arteries. Microdissection specimen

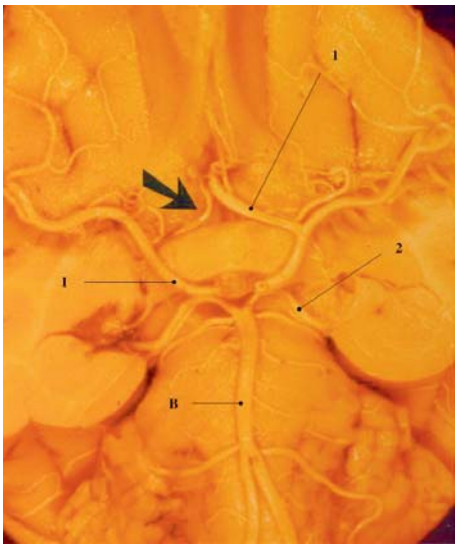

Figure 2. Arterial circle of the *Cercopithecus aethiops* brain base: Internal carotid artery (I), anterior cerebral artery (1), posterior cerebral artery (2) and basilar artery (B). Hipoplastic right anterior cerebral artery (arrow). Microdissection specimen

Description and analysis of the differences of the measured lengths of the arterial ring of the brain base of the GA-monkey have shown the following:

– Boundaries of the interval of variation (min, max) for values of length of the anterior segment are 7.00 mm and 12.00 mm for the left side; 7.25 mm and 11.50 mm for the right side; and 8.15 mm and 23.50 mm for the entire AC anterior segment. The boundaries of the interval of variation (min, max) for the values of

length of the posterior segment are 6.25 mm and 10.10 mm for the left side; 5.95 mm and 11.25 mm for the right side; and 12.20 mm and 21.00 mm for the entire posterior segment (Table 1).

Table 1. Descriptive statistical parameters for the length values

Segment	Side	Statistical parameters		
		$\bar{x}$	sd	cv (%)
Anterior	Left	8.86	1.25	14.11
	Right	8.86	1.30	14.67
	Total	16.72	3.72	22.25
Posterior	Left	8.82	0.89	10.09
	Right	8.92	1.19	13.34
	Total	17.77	2.01	11.31

n=20

– Values of the measured lengths are homogenous (cv<30%) in all of the 6 statistical series (anterior left segment, anterior right segment, entire anterior segment, i.e. total; posterior left segment, posterior right segment, entire posterior segment, i.e. total) (Table 1).

– Arithmetical means of the lengths of the left and right sides of the anterior segment are identical, while the arithmetical mean of the left side was less than the arithmetical mean of the right side of the posterior segment. Arithmetical mean of the length of the left side of the anterior segment is greater than that of the same-sided posterior segment, while the arithmetical means of the right side and the entire anterior segment are less than the arithmetical means of the same categories of the posterior segments (Table 1).

– However, the values of length are not significantly different according to gradation (anterior, posterior) of factor A (segment). Values of length do not differ significantly according to gradation (left, right) of factor B (side). Simultaneous influence of factor A (segment) and B (side) on the length is not significant (Table 2).

– Although the arithmetical mean of total lengths of the posterior segment (17.77 mm) is greater than the arithmetical mean of the total lengths of the anterior segment (16.72 mm), the difference is not significant (Table 2).

Table 2. The significance of the difference in length values according to gradation of factor segment (anterior A_1, posterior A_2) and side (left B_1, right B_2)

Factor	Statistical parameters		Significancy
	F	P	
A	0.002	0.962	$p > 0.05$
B	0.033	0.856	$p > 0.05$
AB	0.037	0.849	$p > 0.05$

Total (anterior – posterior): $t = -1.103$; $df = 38$; $p = 0.277$; $p>0.05$

Description and analysis of the differences of measured diameters of the arterial circle of the brain base of the GA-monkey has shown the following:

– The boundaries of interval of variation (min, max) for the diameter of the anterior segment are 1.75 mm and 2.90 mm for the left side; 1.60 mm and 2.70 mm for the right side; 3.50 mm and 5.60 mm for the entire anterior segment. Boundaries of interval variation (min, max) for the values of diameter of the posterior segment are 1.35 mm and 1.80 mm for the left side; 1.35 mm and 1.80 mm for the right side; 2.75 mm and 3.65 mm for the entire posterior segment (Table 3).

Table 3. Descriptive statistical parameters for the diameter values

Segment	Side	Statistical parameters		
		$\bar{x}$	sd	cv (%)
Anterior	Left	2.13	0.26	12.21
	Right	2.04	0.27	13.24
	Total	4.17	0.49	11.75
Posterior	Left	1.63	0.13	7.98
	Right	1.61	0.13	8.07
	Total	3.24	0.25	7.72

n=20

– The values of the measured diameter are very homogenous (cv<30%) in each of the 6 statistical series (anterior left segment, anterior right segment, entire anterior segment, i.e. total; posterior left segment, posterior right segment, entire posterior segment, i.e. total) (Table 3).

– Arithmetical means of the diameters, of the anterior and of the posterior segment, are greater on the left than on the right side. Arithmetical means of each category of the anterior segment (left, right, and total) are greater than the arithmetic means of the corresponding category of the posterior segment (Table 3).

– Values of diameters differ with high significance according to the gradation (anterior, posterior) of factor A (segment). Arithmetic mean of diameters of the anterior segment (A1) differs with a high significance from the arithmetic mean of the diameters of the posterior segment (A2), on left (B1) as well as on right (B2) side (F_{dL}=58.139; F_{dD}=43.000). The values of diameters do not differ, however, significantly according to gradations (left, right) of factor B (side). Simultaneous influence of factor A (segment) and factor B (side) on the diameter is not significant (Table 4).

– Arithmetical mean value of diameters of the entire anterior segment is greater with a high significance than the arithmetical mean value of diameters of the entire posterior segment (Table 4).

Table 4. The significance of the difference in diameter values according to gradation of factor segment (anterior A_1, posterior A_2) and side (left B_1, right B_2)

Factor	Statistical parameters		Significancy
	F	p	
A	102.194	0.000	$p < 0.01$
B	1.344	0.250	$p > 0.05$
AB	0.611	0.437	$p > 0.05$

Total (anterior – posterior): $t = 7.651$; $df = 38$; $p = 0.000$; $p<0.01$

DISCUSSION

Literature data indicates similarities of certain segments of the brain of the African green monkey to that of the human brain in the complexity of the cytoarchitecture of certain segments of the cerebral cortex (Teofilovski, 1984), as well as in the phenomenon of left/right asymmetry (Teofilovski *et al*., 1983; 1986; Teofilovski-Parapid *et al*., 1991). Our result also reveal similarities of the arterial circle of the brain base of the GA-monkey with the Willis circle in humans, as described by those who state that in humans it is made of paired *aa.cerebri posteriores*, *aa.communicantes posteriores*, terminal segments of *aa.carotis internae*, *aa.cerebri anteriores* and unpaired *a.communicans anterior*, and without partaking of *aa.cerebri mediae* (Peli, 1902), as stated by Malobabić *et al*. (2000). In the morphological sense, the difference between arterial circle in humans and GA-monkey is present in the anterior segment of the circle where in the GA-monkey *aa.cerebri anteriores* are joined into *a.cerebri anterior communis*. This finding is also found in the Rhesus monkey (Kapoor *et al*., 2003). In humans it is described either as a variation, since it is asymptomatical, or as an anomaly, since it is accompanied by a greater incidence of cranial hemorrhages and ischemic strokes (Batujeff, 1889; Servida and Mortillaro, 1962). Other than this, L/R asymmetry, although not significant, undoubtedly is present on the arterial circle of the brain base of the GA-monkey as well. In our series the arithmetical means of diameters of its anterior as well as posterior segments were greater on the left than those on the right side. The revealed L/R asymmetry of the blood vessels of the brain base may represent the basis of the earlier described asymmetry of its sulcar and gyral scheme (Teofilovski *et al*., 1983; 1986; Teofilovski-Parapid *et al*., 1991).

Communicating arteries are in humans frequently the site of congenital anomalies and *a.communicans posterior* is in the front of these (Hartkamp *et al*., 1999; Macchi *et al*., 2002). It may be missing or be afunctional. We have had a case of a hypoplastic *a.communicans posterior* in one of our monkeys.

Analysis of the collected numerical data has indicated a certain difference in dimensions of the anterior and posterior segment of the arterial circle in the GA-monkey. It has been established that the posterior segment was, although insignificantly, longer than the anterior, but the anterior had significantly greater

external diameter. Therefore, it can be expected that the flow speed of blood is different in them, i.e., greater in the posterior segment. These differences could possibly add to a better understanding of the various representations of anomalies in the human's circle of Willis. In humans, extensive studies have shown that the anomalies are more often located on the posterior segment of the Willis' circle. It is assumed that the cause of this is the flow speed in the posterior segment of the Willis' circle (Hartkamp *et al*., 1999). In the light of this, in the past few years, attention has been drawn to the disease "Moyamoya", which has first been noticed in Japan, but today is described in other regions of the world too (Yamada *et al*., 1995; Kawaguchi *et al*., 1996). It is characterized by progressive occlusion of the arteries in the circle of Willis and other various anomalies. Although a gene has been located on the chromosome 17q25 and the hereditary component is undoubtedly present, its etiopathogenesis has not been completely cleared, and there are indications that it is caused by metabolic stress in certain autoimmune disorders.

CONCLUSIONS

Our research of the brain base arteries of the African green monkey (*Cercopithecus aethiops*) has shown the following:

– On the base of their brain there is an arterial circle which is made of right and left internal carotid arteries (*a.carotis interna dextra et sinistra*) and their branches the right and left anterior cerebral arteries (*a.cerebri anterior dextra et sinistra*), and terminal branches of the basilar artery (*a.basilaris*) – right and left posterior cerebral arteries (*a.cerebri posterior dextra et sinistra*). The anterior cerebral arteries join into anterior common cerebral artery (*a.cerebri anterior communis*);

– Macroscopically there is an evident left/right asymmetry (unilateral hypoplasia and fenestration) of the anterior cerebral artery and posterior communicating artery, which has been noted;

– Left/right asymmetry of the lengths and diameters of arterial segments was not statistically significant;

– The posterior segment is on average, although statistically insignificant, longer than the anterior segment of the arterial circle of the brain base, while the anterior segments in every category (left, right, total) have significantly greater diameters than the posterior segment;

The African green monkey with the described similarities of the brain base arterial circle, which correlates to that of the humans, may represent a suitable experimental animal model in complex cardiovascular research.

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ARTERIJSKI KRUG BAZE MOZGA ZELENOG AFRIČKOG MAJMUNA

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SADRŽAJ

Istraživanja su obavljena na 20 mozgova Zelenog afričkog majmuna (*Cercopithecus aethiops*) čije je moždano vaskularno korito injicirano Latexom. Posle fiksacije u 10% rastvoru formalina, arterije baze mozga su podvrgnute stereomikroskopskoj disekciji i merenju. Ustanovljeno je da na bazi mozga ovog majmuna postoji arterijski prsten koji formiraju, napred, završni delovi obe *aa.carotis internae* i njihove grane *a.cerebri anterior dextra et sinistra*, a pozadi, završne grane *a.basilaris*, *a.cerebri posterior dextra et sinistra*, kao i *a.communicans posterior dextra et sinistra*. U 3 slučaja je uočena morfološka L/D asimetrija arterija baze mozga – hipoplastična *a.cerebri anterior dextra*, *hipoplastična a.communicans posterior dextra* i fenestracija *a.cerebri anterior dextrae*. Na osnovu izmerenih vrednosti dužina i dijametara arterija koje čine arterijski prsten baze mozga izračunati su dužina i dijametar prednjeg (levo, desno, ukupno) i zadnjeg segmenta. Merenja su ukazala na blagu L/D asimetriju segmenata arterijskog prstena, kao i na razlike izmedju njegovog prednjeg i zadnjeg segmenta. Međutim, konstatovana L/D asimetrija nije bila statistički značajna ($p>0.05$) ni za vrednosti dužina ($F_B=0.033$; $p=0.856$) ni za vrednosti dijametara ($F_B=1.344$; $p=0.250$). Prednji i zadnji segmenti prstena ne razlikuju se značajno ($p>0.05$) po dužini ($F_A=0.002$; $p=0.962$; $t=-1.103$; $p=0.277$). Dijametri prednjeg segmenta arterijskog prstena baze mozga, visokoznačajno su veći ($p<0.01$) od dijametara zadnjeg ($F_A=102.194$; $p=0.00$; $F_{dL}=58.139$; $F_{dD}=43.000$; $t=7.651$; $p=0.00$). Zeleni afrički majmun sa opisanim sličnostima arterijske šeme baze mozga sa odgovarajućom šemom kod ljudi, može predstavljati pogodnu eksperimentalnu životinju u kompleksnim istraživanjima kardiovaskularnog sistema.