

**BLOOD SUPPLY OF THE INSULAR CORTEX OF *CERCOPITHECUS AETHIOPS*: ANOTHER APPROACH TO ESTABLISH NON-HUMAN PRIMATE MODEL FOR ADDICTION**

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*The non-human primate model has already been applied in studies about alcoholism in the last decade, demonstrating a high confidence level of similarities between humans and non-human monkeys. Authors indented to establish the definitive model for addiction by investigating the insular blood supply in Cercopithecus Aethiops. In 23 out of 24 Latex injected adult brains, the insula received blood supplied by the M2 segment of the middle cerebral artery (MCA). The cortex was supplied by two separate trunks, while perforating branches originating directly from MCA, individually, or together with lateral striate (lenticulostriate) arteries. Their average caliber was about 120 µm, and the number of its branches ranged from 2 to 9. No left – right asymmetries have been recorded. Similarities with the human vascular pattern have been studied.*

*Key words: insula, blood supply, Cercopithecus Aethiops, addiction, non-human primate model*

**INTRODUCTION**

The non-human primate model has already been applied in the studies about alcoholism in the last decade, demonstrating a high confidence level of similarities between humans and non-human monkeys (Higley and Linnoila, 1997; Higley and Bennett, 1999) in both morphological and behavioral modes. The anatomical comparability between branches of the middle cerebral artery (MCA) in humans and *Cercopithecus Aethiops* (CA) has been reported long time ago (Bollert and Swindler, 1968) and subsequently almost axiomatically accepted. Previous studies revealed the applicability of the prefrontal circulation on non-human primate model for schizophrenia, despite some morphological differences between CA and humans (Filipović *et al.*, 2005).

The insular cortex in CA has a role in vocal alarm and food advertisement calls that involve an emotional component (Jarvis, 2006). In humans, recent

investigations are involving the insular cortex in semantic ("tip – of – the tongue") mistakes, but also outline the ambiguousness of the impact that it has in schizophrenia and cocaine addictions (Makris *et al.*, 2006; Duncan *et al.*, 2007; Saze *et al.*, 2007; Shafto *et al.*, 2007).

#### MATERIAL AND METHODS

The investigation was performed on brains of 24 healthy, non-infected, decapitated male adult *Cercopithecus Aethiops*, obtained from the Institute of Immunology – "Torlak". Euthanasia by application of Kethonal® (Galenika, Beograd), was entirely congruent to the National Guidelines on Experiments on Laboratory Animals. The head and neck complexes were immediately after decapitation injected with liquid rubber (Latex®) under 140 mm Hg pressure, through a baby system for catheterization placed in the common carotid artery and were preserved in 10 % formaldehyde solution. Within an one – month period, fixed brains were removed out of the cranial cavity and kept in a form of "floating fixation" (tied to the carrier after submersion in order to prevent deformations) in a solution mixture of 4 % formaldehyde and 70 % ethanol for one month. Finally, fixed brains underwent a microdissection under stereo magnifying glass. The brain tissue of the temporal lobes was removed in order to expose the insular segment of the middle cerebral artery (M2) for exploration. The number, origin, diameters of the insular branches, and middle cerebral artery/insular branches caliber ratio on each hemisphere were revealed. The diameters of the blood vessels were obtained by calibrated ocular micrometer with a measurement error of 20 µm, at the very site of branching. In statistical evaluation, descriptive statistics (central tendency measures: mean and mode, standard deviation - SD, minimum, maximum) was applied, while interhemispheric differences were verified by Student's t-test for paired samples, and chi-square test for non-parametric values. The testing was performed on 95 % probability level.

#### RESULTS

In 23 out of 24 brains, insular branches (IB) originated in M2 segment, form the ventral and dorsal terminal trunks of the middle cerebral artery (MCA). Their number was constant, consisting of two arterial trunks, namely the anterior and posterior trunk (AT and PT, Figure 1), dividing separately from the M2 segment of the middle cerebral artery, per hemisphere. In one they originated from the "early branching" of MCA (Figure 2). From the branching point both mother arteries divided to a number of branches supplying the anterior and posterior insular cortices. The number ranged from two to nine (Table 1). The insular perforating arteries arose from the distal portion of the MCA, mostly in the common trunk that, subsequently, branched to a variable number of arteries. On one specimen the anterior common trunk was joined with the lateral striatal arteries. Results are shown in Table 1.

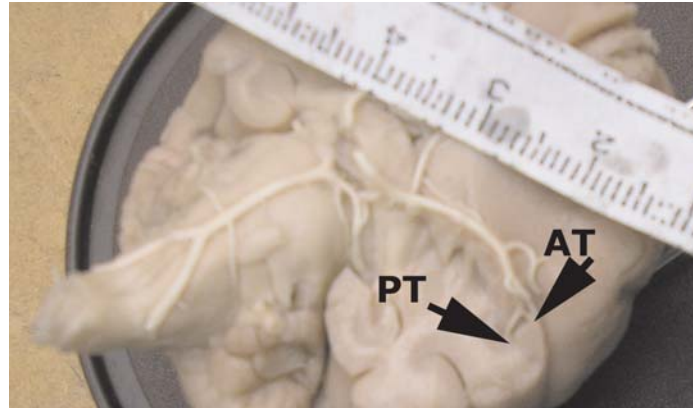


Figure 1. The most common vascular pattern for insula. Anterior trunk (AT) and posterior trunk (PT) rising from the middle cerebral artery.



Figure 2. The origin of the anterior trunk from the "early branching" from MCA.

Table 1. Number of branches from the main trunks supplying the insular cortex

	Left hemisphere		Right hemisphere	
	Anterior trunk	Posterior trunk	Anterior trunk	Posterior trunk
Minimum	2	2	2	1
Maximum	9	6	4	3
Mod	3	3	2	2

$\chi^2=0.06$   $df=1$ ,  $p>0.05$

In order to make an adequate comparison with human insular arborization, scores between the common trunk and its daughter branches and parent trunk of MCA, and their distribution were calculated and shown in Tables 2 and 3. The trend of left – right asymmetry remained in the evaluated scores, as well.

Table 2. Mean values ( $\pm$  SD) and ranges of the obtained common trunks diameters (in micrometers)

	Left hemisphere		Right hemisphere	
	Anterior trunk	Posterior trunk	Anterior trunk	Posterior trunk
Minimum	90	80	70	100
Maximum	230	220	180	170
Mean $\pm$ SD	124 $\pm$ 22.8	132 $\pm$ 19.7	116 $\pm$ 19.3	114 $\pm$ 13.8

t – test = 0.411, df = 23, p>0.05

Table 3. Insular common trunk/MCA parent trunk scores

	Left hemisphere		Right hemisphere	
	Anterior trunk	Posterior trunk	Anterior trunk	Posterior trunk
Minimum	0.09	0.08	0.05	0.06
Maximum	0.15	0.16	0.14	0.14
Mean $\pm$ SD	0.12 $\pm$ 0.02	0.13 $\pm$ 0.014	0.124 $\pm$ 0.009	0.123 $\pm$ 0.01

t – test = 0.387, df = 23, p>0.05

## DISCUSSION

The vascular pattern of the insula in African Green monkeys (*Cercopithecus Aethiops*) is rather different than the one in humans: we have obtained only two common trunks which supply the insular cortices, while human brain arteries for insular cortices nourishment arose from prefrontal, precentral, angular and temporooccipital branches of the MCA (Tanriover *et al.*, 2004). The presence of the perforating branches for the insula has also been reported (Türe *et al.*, 2000; Tanriover *et al.*, 2004). The total number of human insular branches ranged from 77 to 112 (Türe *et al.*, 1999; 2000) and in CA this number does not surpass nine cortical branches per hemisphere. Certain parallels, however, can be drawn: in humans, the blood supply derives mostly from the M2 segment, as it is in CA. Further, it is divided into the anterior and posterior compartments; in CA we have revealed most frequently anterior and posterior common insular trunks in the cortical blood supply. Perforating branches, nonetheless, could have a common origin with the lateral lenticulostriate arteries, as we have obtained in one isolated specimen, or they could arise separately, as it was seen in most of our cases (Türe

*et al.*, 2000). Finally, scores between calibers of insular branches in humans and CA are almost similar (approximately 1:10 in humans, and 1:8 in CA).

Reduction in the blood flow in the insular region has been remarked in the temporal lobe in epileptic patients (Giovancchini *et al.*, 2007) and in early psychotic episodes (Achim *et al.*, 2007), but also in alcohol and cocaine addiction, in humans and experimentally induced dependency in laboratory animals (Wallace *et al.*, 1996; Gottschalk and Kosten, 2004; McBeth *et al.*, 2005). Some authors are discussing the increased blood flow in the insular region during alcohol craving episodes. Oppositely, the insular cortex in nicotine addiction appears to be intact and consecutively the recovery process of the nicotine addicts is supposed to be easier (Olbrich *et al.*, 2006; Naqvi *et al.*, 2007). The impulsivity in cocaine addicts could also be based on the damaged insular cortex and impaired blood flow, hence the negative influence of cocaine hydrochloride on cerebral blood flow is known for years. The insular cortex shows activation during impulsive reactions (Arce and Santisebastian, 2006).

#### CONCLUSION

Insula in the *Cercopithecus Aethiops* monkey is supplied by the branches of the middle cerebral artery, arising most frequently from the M2 segment, according to the MCA pathway division in humans. Certain similarities between human and CA blood supply have been obtained, such as the origin, distribution and the daughter/mother arteries calibration scores. In conclusion, we have to say that insular blood supply in CA expresses some differences if compared to humans, but regarding the source and the distribution of insular branches the use of CA insular cortex for addictions studies, as the non-human primate model, is acceptable from the anatomical point of view.

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**VASKULARIZACIJA KORTEKSA INSULE *CERCOPITHECUS AETHIOPS*-a –  
DRUGAČIJI PRISTUP FORMIRANJU NEHUMANOIDNO-PRIMATSKOG MODELA  
ZA IZUČAVANJE BOLESTI ZAVISNOSTI**

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SADRŽAJ

Nehumanoidni primatski model je u poslednjoj dekadi primenjivan u studijama o alkoholizmu i one su ukazale na visok stepen povezanosti pojava kod ljudi

i nehumanoidnih majmuna. Svrha ovih ispitivanja je bila da se dâ doprinos formiranju definitivnog modela za izučavanje bolesti zavisnosti. U 23 od 24 ubrizgana mozga insulu su vaskularizovala dva stabla poreklom iz M2 segmenta srednje moždane arterije (*a. cerebri media* – ACM), dok su u jednom slučaju grane poticale iz tzv. "ranog grananja" ACM. Perforantne grane za ishranu insule odvajale su se bilo zajedničkim stablom ili zasebno, ali uvek u delu iz koga su se odvajale lentikulostrijalne arterije. Prosečan promer arterijskih stabala iznosio je na levoj hemisferi: prednje stablo  $124 \pm 22,8 \mu\text{m}$ , zadnje stablo  $132 \pm 19,7 \mu\text{m}$ ; dok su na desnoj hemisferi vrednosti bile  $116 \pm 19,3 \mu\text{m}$ , za prednje, odnosno  $114 \pm 13,8 \mu\text{m}$  za zadnje stablo, dok je broj grana varirao od 2 do 9. Nisu uočene bilo kakve levo – desne razlike u dobijenim rezultatima. U radu su razmatrane sličnosti i razlike sa načinom vaskularizacije u ljudi.