Contribution to the Morphological Study of the Cerebral Arterial Circle (Circle of Willis) of Rats

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Abstract: In this paper the Circle of Willis (CW) was morphologically studied in 87 Wistar rats in the Ribeirão Preto Medical School. The vessels of the brain were contrasted with colored latex, just after the euthanasia of the animal. After removal of the brain from the skull, the CW and their main components were dissected under microsurgical vision, schematically reproduced and photographed for analysis. The CW presented patterns and variations, which were more or less constant. The constant patterns were: 1. its components have anastomosis among them, making the circle a closed structure; 2. the middle cerebral artery (MCA) and the nasal olfactory artery (NOA) are patent vessels; 3. the origin of the superior cerebellar arteries (SCbAs) ocurred as terminal branches of the basilar artery (BA); and 4. the A2 segment of the anterior cerebral artery (ACA) is an azygos vessel. Among the not constant but frequently observed patterns: 1. the variation in the origin of the posterior cerebral arteries (PCAs), and the presence of the thalamoperforating arteries (TPAs); 2. the presence of the anterior choroidal artery (AChA), and of the corticoamygdaloid artery (CoAmA); 3. the singleness in the origin of the MCAs and of the A1 segments of the ACA; 4. the presence of anastomosis between both A1 segments of the ACAs; 5. the origin of the ventral olfactory artery (VOA) from the A1 segment of the ACA inside the longitudinal cerebral fissure; and 6. the junction of the A1 segments to form an A2 azygos vessel, inside the longitudinal cerebral fissure, near the genu of the corpus callosum. The results of this paper were in accordance with the most part of the data found in the literature, outstanding the finding of well-defined patterns for the anterior and posterior regions of the CW of rats, as well as, the main variations of their components, demonstrated through schemata and photographies.

Keywords: Circle of Willis, Vascular Anatomy, Rat Brain.

1. INTRODUCTION

The human cerebral ischemia is among the events that most frequently affects the species. Due to ethical limits in *in vivo* studies for understanding this phenomenon, laboratory animals have been used as experimental models. The advantages of these are the best control on the biological variables, the invasive methods can be used more freely and the results are measured accurately [1]. The rat is one of the most used animals due to easy obtainment and handling, the homogeneity of the animals of the same breed, and is very similar from the aspects of anatomy and cerebrovascular physiology with other animals, including humans [2]. Despite some differences, the rat cerebral arterial circle – Circle of Willis (CW) – closely resembles that of a human [2,3]. It allows vast communication between the right and left carotid systems and the vertebrobasilar system. In the rat, as well as in other primitive mammals, the carotid system represents the most important source of arterial blood supply to the brain, and the vertebrobasilar system contributes to the supply of the brainstem [4]. The rat presents the anterior cerebral artery (ACA), the middle cerebral artery (MCA) and the posterior cerebral artery (PCA). The ACA and MCA arise from the internal carotid artery (ICA), while the PCA originates from the vertebrobasilar system [5-8]. The left and the right carotid systems communicate through the ACA (Figure **1**) [3,5,6,9-11]. The carotid system communicates bilaterally with the vertebrobasilar system through the posterior communicating artery (PCoA) [5-8]. The nomination of vessels that communicate the carotid and vertebrobasilar systems varies according to the authors. Greene [9] nominated PCoA the segment joining the ICA and the superior cerebellar artery (SCbA). Tamura *et al*. [3] and Zea Longa *et al*. [11] considered the PCA as a direct branch of the ICA, and PCoA as the segment that connects the PCA to the vertebrobasilar system. The PCA arises from either the SCbA or the bifurcation of the basilar artery (BA), heading superiorly and slightly laterally, passing posteriorly and laterally to the pituitary stalk, curving more laterally and following a winding path until it reaches PCoA, closing thus the caudal part of the circle (Figure **1**). It then curves around the stalk of the brain and follows dorsally along the edge of the cerebellar

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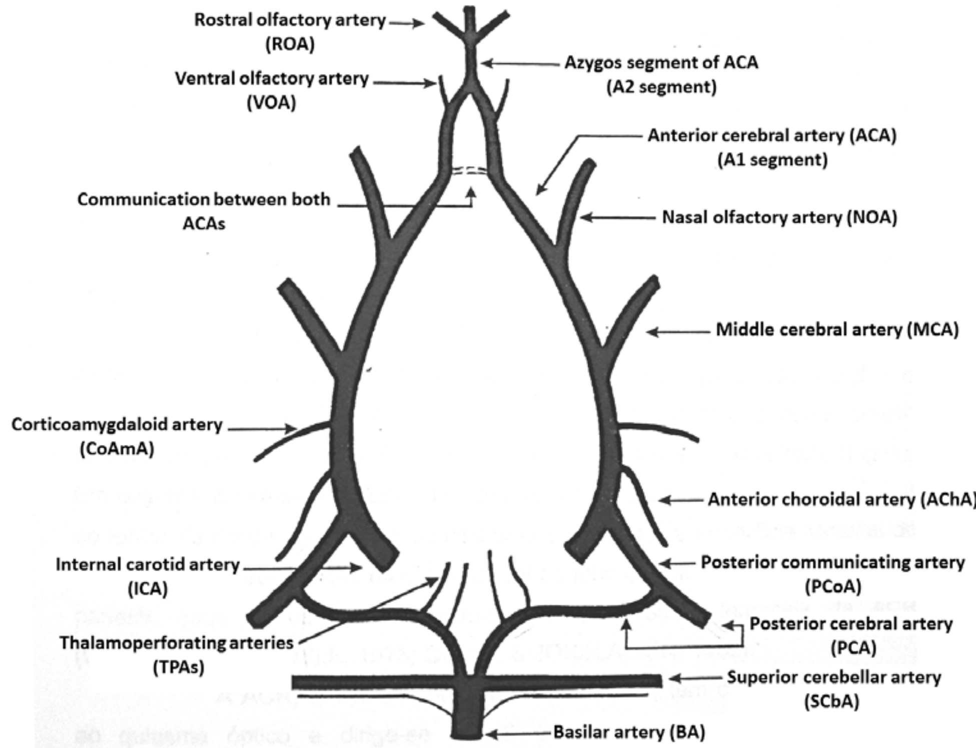

Figure 1: General scheme of the CW of the rat.

tentorium and will irrigate the tentorial surface of the hemisphere and the medial and lateral surfaces of the occipital lobe [7,9,12]. The PCoA emerges from the ICA, laterally to the pituitary stalk, and bends caudally to find the PCA. The anterior choroidal artery (AChA) originates from the ICA, distal to the emergence of PCoA (Figure **1**). It proceeds in the groove between the temporal lobe and the brainstem, curving dorsally around it. After it reaches the lateral ventricle, the AChA protrudes in the medial side of the ventricle, forming the choroid plexus, without actually penetrating the cavity of the ventricle. It participates in the formation of the III ventricle choroid tela, similar to the lateral ventricle [9]. The corticoamygdaloid artery (CoAmA) originates from the lateral wall of the ICA, superiorly to the posterior border of the optic chiasm. Follows a path on the caudal piriform cortex and its lateral branches, anastomoses with branches of the rhinal artery. MCA, the larger of the terminal branches of ICA, originates laterally to the infundibulum, and is directed obliquely, in rostrolateral direction, on the lateral surface of the olfactory cortex, giving rise to several branches to the piriform cortex. In the lateral olfactory tract level, the MCA emits a branch in rostral direction, the corticostriate artery (CoStA), which irrigates the rostral portion of the piriform cortex and lateral olfactory tract. The CoStA corresponds to the lenticulostriate arteries in the human brain [8,12]. After the origin of the CoStA, MCA curves on the lateral surface of the hemisphere, and branches variably forming vessels that are usually represented by the rostral, middle and caudal groups. The irrigation of the medial part of the caudate-putaminal complex is supplied by the recurrent arteries of Heubner, which originate from the ACA [3]. The nasal olfactory artery (NOA) [7], or simply olfactory artery [6,8], is a calibrous branch of ACA that emerges approximately at the midway between the origin of the ACA and its communication with the contralateral homonymous. A vessel of similar origin and destination can be found in human embryos, but does not persist into adulthood [7,8]. The ethmoid artery replaces the NOA in mammals that do not have this vessel [7,8]. The ACA, one of the terminal branches of the ICA, originates laterally to the optic chiasm runs medially and superiorly, as if it were a continuation of the ICA. Ahead of the optic chiasm, it penetrates the longitudinal cerebral fissure, and after a short route unites with the contralateral homonymous, forming an azygos vessel [7,8]. This vessel follows the longitudinal cerebral fissure, curving dorsally around the genu of the corpus callosum, becoming the pericallosal artery (PA), also an azygos vessel. At the level of this transition, it sends frontal cortical branches and runs on the cingulate cortex and on medial portions of the frontal cortex from both hemispheres to finally anastomoses with the medial terminal branches of the MCA [7,8]. The PA follows caudally, originating the caudal internal frontal artery and the retrosplenial artery, which are terminal branches that supply the retrosplenium and occipital cortex. Terminal anastomosis are evident between these branches and the caudal branches of the MCA. There are few morphological studies in the rat that address the anatomy of CW, so the objective of this study was to perform and review its mesoscopic morphological description.

2. METHODS

Eighty seven Wistar rats, adult, male, with a mean weight of 328.5g (range 285 to 375g) were used. The animals were provided by the Central Animal Facility of the Campus of Ribeirão Preto, University of São Paulo. The experiments were initiated after approval by local Ethics Committee of Animal Research. After inhalational anesthesia with ether, the animals were submitted to thoraco-phreno-laparotomy. The abdominal aorta, the portal vein and inferior vena cava were clamped simultaneously. Venous blood was drained by incision in the right atrium, and the left ventricle was punctured with a calibrous needle for perfusion with physiological saline solution 0.9%, ceasing when the perfusion fluid drained through the right atrium presented pink coloration. The animal was then euthanized with an intraventricular injection of 10ml of formaldehyde 10%. Immediately after euthanasia, 15ml of a mixture containing latex and formaldehyde 10% were injected, interrupting the injection when the greenish color arise in the skin or in the eye of the animal. After staining, the animal was decapitated, his head being preserved in formaldehyde 10% for at least 72 hours, until the removal of the brain. For this procedure, was used microsurgical technique aiming at the preservation of the blood vessels at the skull base. The optic, oculomotor and trigeminal nerves had to be sectioned to allow removal. The ICA was always sectioned before its first branch, the PCoA, and in all cases the CW was preserved. For each brain dissected, every segment components of the CW were outlined in a drawing, as well as all visualized branches arising from each segment, with special attention to the shape and size of each vessel. The nomenclature used was based on Illustrated Veterinary Anatomical Nomenclature [13] and eventually the Nomina Anatomica [14]. All brains were photographed at baseline incidence, in panoramic mode and highlighting the rostral and caudal regions of the CW.

3. RESULTS

3.1. Superior Cerebellar Arteries (SCbAs)

Were identified in all 87 cases. They were originated from the bifurcation of BA, and extended laterally and dorsally in the groove between the pons and midbrain. They were nearly symmetrical in shape and caliber on both sides. In 67 cases (77.1%) there was a small caliber artery emerging from BA on the left side; on the right side, this small artery was seen in 61 cases (70.11%), possibly a ventral pons artery.

3.2. Posterior Cerebral Arteries (PCAs)

Were studied in 84 pieces. Their origin in the vertebrobasilar system occurred at various locations (Figure **2**). In 39 cases (46.42%) the origin of the two PCAs was found in the bifurcation of the BA, being the two together, one next to the other, but coming from separate trunks (Figure **2A**). In a second pattern the left PCA emerged from the bifurcation of the BA, and the right PCA from the ipsilateral SCbA (13 cases – 15.47%) (Figure **2B**). Both PCAs originated from the left SCbA in 11 cases (13.9%), and in 11 other cases both originated from the right SCbA (Figure **2C** and **2D**). In 8 cases the right PCA came from the bifurcation of the BA and the left PCA came from the ipsilateral SCbA (9.52%) (Figure **2E**). Both PCAs originated from the bifurcation of the BA in a single trunk in 2 cases (2.38%) (Figure **2F**), and in 1 case (1.19 %) each PCA originated from the ipsilateral SCbA, close to the bifurcation of the BA (Figure **2G**). The initial portion of the PCA, the one that goes from its origin to the anastomosis with the PCoA, was always thinner than the distal portion.

3.3. Thalamoperforating Arteries (TPAs)

They were analyzed in 81 pieces. There were variations in number and caliber, and some of them were very thin, forming a kind of network. The arteries of which the visibility was unquestionable were counted. The left PCA originated the TPAs in 67 brains (82.71%), and the right PCA in 62 (76.54%). Usually there was one artery on each side, and eventually one side had two arteries. In 14 brains on the left, and in 19 on the right, such branches were not found as an individual vessel, but a network of small vessels.

3.4. Posterior Communicating Arteries (PCoAs)

These arteries were observed in all 87 cases. They were seen as thick vessels, and represent the first

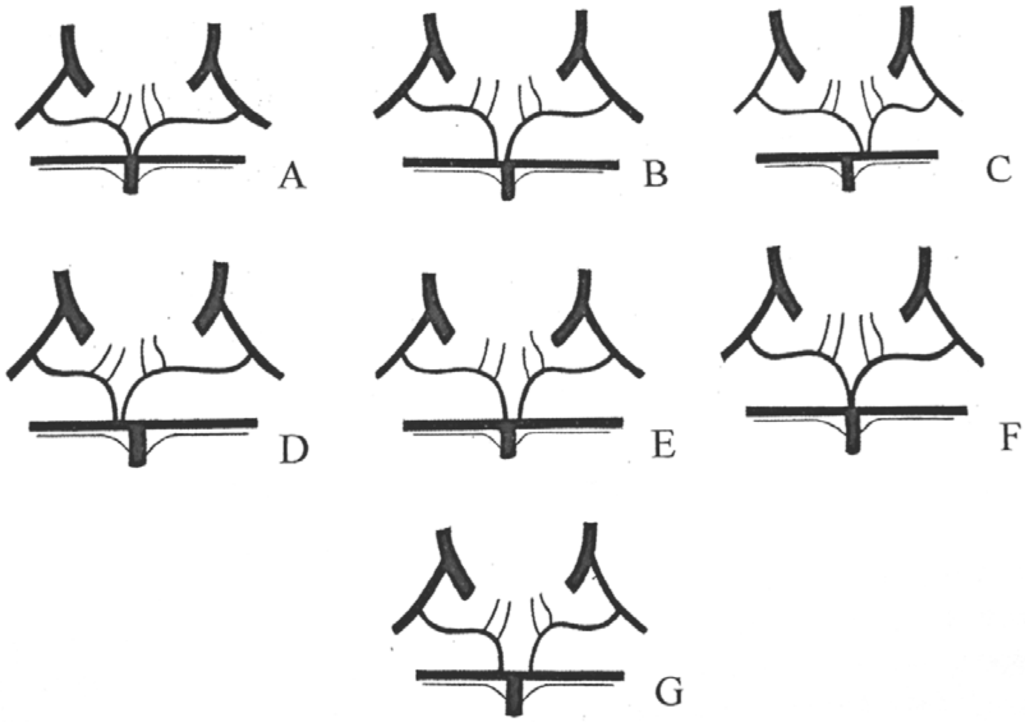

Figure 2: Scheme of the origins of the PCAs in the vertebrobasilar system.

intracranial branch of the ICA. They run caudally and slightly to the side until they reach the PCA (Figure **3**).

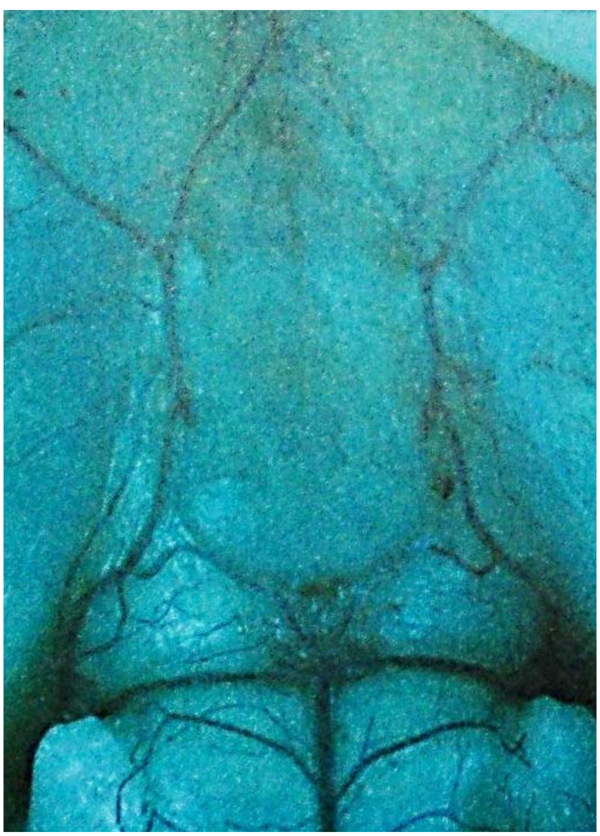

Figure 3: Photograph of the CW evidencing the PCAs, PCoAs, left AChA, origin of left MCA from 2 branches, right CoAmA, and NOA.

3.5. Anterior Choroidal Arteries (AChAs)

They were observed in all 87 specimens. In 71 cases (81.60%) they were seen bilaterally, and in 8 cases (9.19%) they were absent. In 5 cases (5.74%) they were only present on the left, and in other 3 cases (3.44%) only on the right. In the cases in which they were present, the AChAs originated from the ICA (Figure **3**), except for three cases (two on the left and one on the right), in which the origin was in the PCoA (Figure **4**).

3.6. Corticoamygdaloid Arteries (CoAmAs)

They were found in 61 brains (70.11%), and had different sizes (Figure **3**). In 35 cases (57.37%) they were bilateral. In 18 cases (29.50%) they were only present on the left, and in 8 cases (13.11%) only on the right. In 5 specimens (8.19%) there was more than one artery on the same side: on the right in 3 cases (4.91%) and on the left in 2 cases (3.27%).

3.7. Middle Cerebral Arteries (MCAs)

In 69 cases (79.31%) they were originated from the ipsilateral ICA from a single branch. In 18 cases (20.68%) the MCA was originated by more than one branch, and those joined to form a trunk; in 9 cases (10.34%) it was originated by two branches on the left (Figure **4**); in 7 cases (8.04%) by 2 branches on the right, and in 1 case (1.15%) it had a double origin on both sides. In 1 case the left MCA was formed by 3 branches. In most cases there was a main branch, larger than the accessory branch, although in some cases the two branches presented similar calibers.

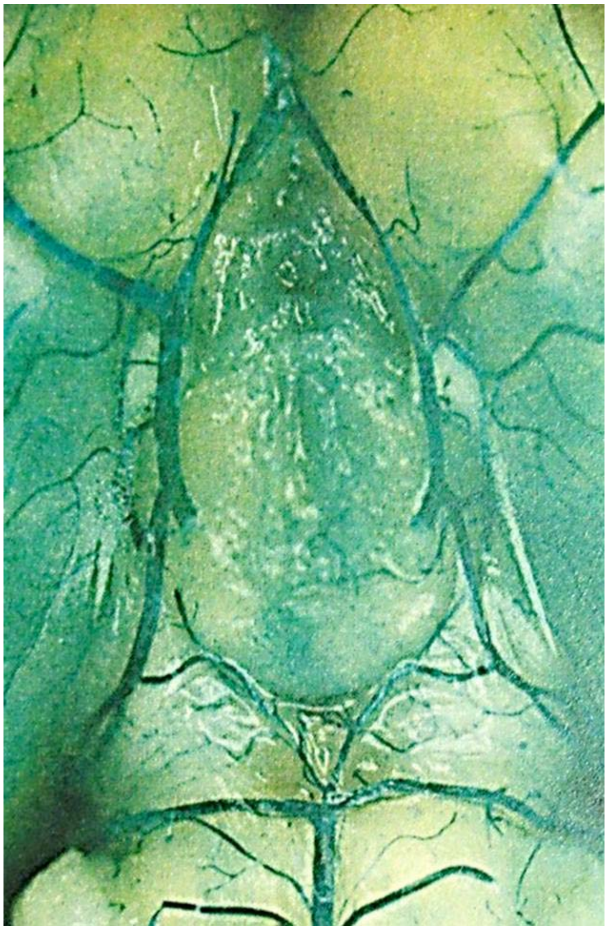

Figure 4: Photograph of the CW evidencing the origin of both PCAs from a single short trunk after bifurcation of the BA, and TPAs from the left PCA.

3.8. Anterior Cerebral Arteries (ACAs)

3.8.1. Nasal Olfactory Artery (NOA)

This artery, also called olfactory artery [6,8], is the most calibrous branch of the ACA. It was seen in 87 cases. It emerged from the initial portion of the ACA, always proximally to its entry in the longitudinal cerebral fissure. It became cranially for a few millimeters (Figure **3**). From that point its study wasn't possible, because it exits the skull through the dorsomedial crivous foramen [6], and it must be sectioned to remove the brain.

3.8.2. Morphology of the A1 Segments

This segment extends from the origin of ACA to the point where it communicates with its contralateral namesake to form an azygos vessel, which is the A2 segment. This point is located inside the longitudinal cerebral fissure, near the genu of the corpus callosum. In 57 cases (65.51%) the A1 segment consisted of a single vessel bilaterally (Figure **3**). In 30 cases (34.48%) part of the segment was duplicated, forming a "buttonhole" image (Figure **5**); in 14 of these cases (16.09%) the duplication was bilateral (Figure **6C**), in 11 cases (12.64%) only on the left, and in 5 cases (5.74%) only on the right. This duplicity was observed in most cases near the point where the A1 segment enters the longitudinal cerebral fissure.

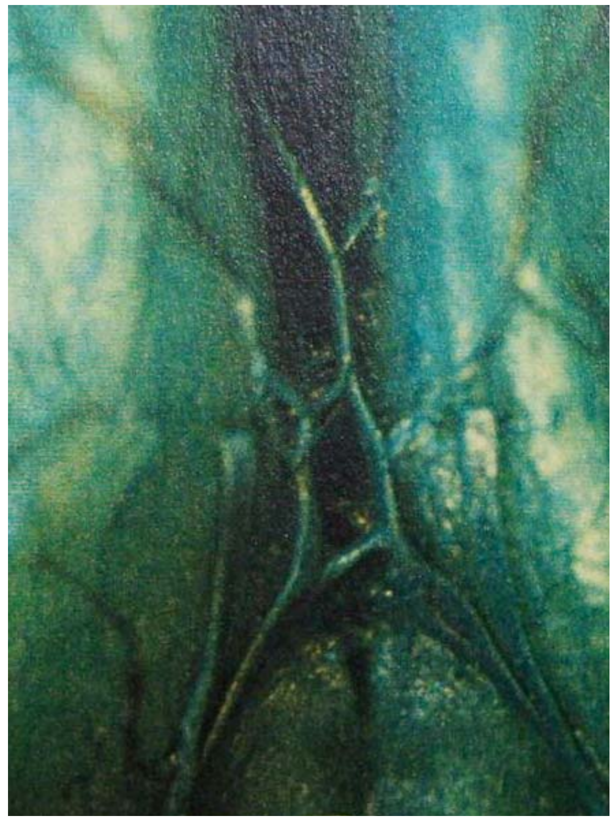

Figure 5: Photograph of the rostral portion of the CW evidencing double left A1, 2 communications between A1, dominant left A1 and right A1continuing as VOA.

3.8.3. Vascular Communications between the A1 Segments

In all of the 87 analyzed cases there were communication points between the right and left A1 segments. In 55 cases (63.21%) there was only one communication in the form of a junction, which occurred inside the longitudinal cerebral fissure (Figure **6A**). In 53 of these cases the point of communication occurred near the genu of the corpus callosum, and in 2 cases the communication occurred at the entrance of the longitudinal fissure, in front of the optic chiasm. In 32 cases (36.78%) more than one communication

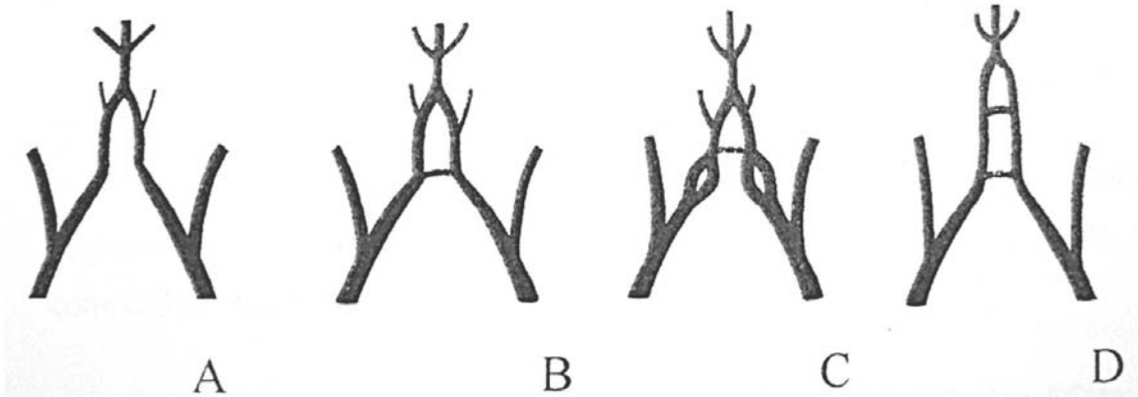

Figure 6: Scheme of the rostral portion of the CW depicting the several types of the A1 segment communications.

points were observed between A1 segments; in 24 cases (27.58%) a communication was observed at the entrance and another in the depths of the longitudinal cerebral fissure (Figures **5** and **6B**); 4 cases (4.59%) had two communications within the fissure (Figure **6C**), and other 4 cases showed a communication at the entrance of the fissure and two others within it (Figure **6D**).

3.8.4. Origin of Branches of the ACA Distal to the Nasal Olfactory Artery (NOA)

The first distal branch of the ACA is the ventral olfactory artery (VOA) [7], which was originated in different places, but always inside the longitudinal cerebral fissure. It emerged from the right A1 segment in 72 cases (82.75%), and from the left A1 segment in 73 cases (83.90%) (Figure **1**). It represented the main continuation of the A1 segment (after lateral communication with its contralateral namesake) in 10 cases on the right (11.49%) (Figure **5**), and in 5 cases on the left (5.74%). It was originated from the A2 segment in 9 cases (10.34%) on the left, and in 5 cases on the right (5.74%).

3.8.5. Place and Manner of Formation of the A2 Segment of the ACAs

The A2 segment was an azygos vessel in the 87 specimens, formed in three different ways: 1) by simple fusion of A1 segments (Figure **6A**) which occurred in 63 cases (72.41%); 2) as a continuation of the left A1 segment after lateral communication with the right A1 segment (Figures **5** and **7A**), observed in 17 cases (19.54%); 3) as a continuation of the right A1 segment after communication with the left A1 segment (Figure **7B**), which occurred in 7 cases (8.04%).

DISCUSSION

In this study was used the manual contrast injection technique, guided by the greenish coloring of the skin or the back of the rat´s eyes. The best specimens for the study of arterial vessels were those that had no such coloring, since in those cases only arteries were contrasted, but not veins. Some findings were consistent in all animals and variations were frequently observed. In all brains it was verified that the CW is a closed structure; the MCA and the NOA are present in

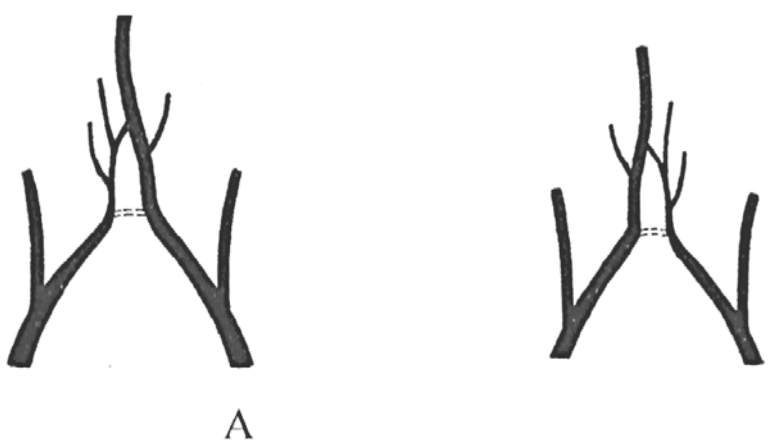

Figure 7: Scheme of the rostral portion of the CW depicting the predominance of the left A1 segment (**A**) and the right A1 segment (**B**), for A2 segment origin.

all specimens; the origin of SCbAs always occurred as a terminal branch of BA, and the A2 segment of the ACA is always an azygos vessel. Some patterns were not consistent in all brains, but drew attention for its high frequency. Many variations were found regarding the place of origin of the PCAs. Our material has revealed that in over half the cases, the place of origin varies (53.58%). The most frequently pattern was the symmetric origin of both PCAs, at the point of the bifurcation of the BA, side by side in separate branches (46.42%). Soares *et al*. [8] found that the most commonly found pattern of the origin of these arteries was in a short common trunk originated in the bifurcation of the BA. These observations probably do not disagree, since the presence or absence of a short common trunk at the bifurcation of the BA after the emergence of the SCbA depends on the standards of the observer. Scremin [15] stated that the PCA commonly originates in the SCbA, but does not quantify this occurrence. Regarding the presence of TPAs in our study these branches could be visualized in 93% of cases, there being greater caliber branch on each side, but eventually two branches can occur on each side or in one side. These branches were originated in the left PCA in 82.71% of cases and in the right PCA in 76.54%. In the other cases (17.29% on the left and 23.46% on the right) was observed a network of small vessels. Scremin [15] also reports that these arteries originate from the PCAs in variable number, usually three on each side, and run rostrally and dorsally until they reach the ventrocaudal region of the thalamus. Regarding the presence of the AChAs, it was possible to identify them bilaterally in 81.60% of cases, originating from the ICAs, distally to the origin of the PCoAs. Moffat [5] and Coyle [7], when describing these vessels, mention the anastomosis between the AChA and the caudal lateral choroid branch, usually originated from the PCA, establishing a deep communication between the carotid and vertebrobasilar systems. The CoAmAs, cortical branches of the ICAs, were found in 70.11% of the brains, being bilateral in more than half of the cases. In almost all cases, a single branch was noticed on each side. They had variable calibers and longitudinal dimensions, but they were all conspicuous. Coyle [7] stated that several branches of the MCA and ICA irrigate the periamygdaloid cortex and the amygdala. Scremin [15] described that the CoAmA originates on the side of the ICA and spreads caudally on the piriform cortex and anastomoses with branches of the rhinal artery. Regarding the MCA, one of the terminal branches of the ICA, it originated by a single branch on each side in 79.31% of the cases, presenting then extensive and variable branching. In 20.69% of cases, the MCA was formed by 2 (94.44%), or 3 branches (one case – 5.56%), and in 17 of these cases the variation was unilateral (56.25% on the left, and 43,75% on the right). Brown [6] observed a duplicity in the formation of the MCA in 13.79% of cases, both branches having approximately equal caliber, and also noted that the duplicity was more common on the right. Wiland [10] found the MCA being formed by two branches in 41.17% cases (17 unilaterally and 4 bilaterally), and 1 case where the MCA was formed by three branches. According to Scremin [15], the MCA runs laterally and dorsally on the olfactory cortex and sends several branches to the piriform cortex. On the level of the lateral olfactory tract, it emits a branch that runs rostrally, the corticostriate artery (CoStA). This vessel supplies the rostral portion of the piriform cortex and the lateral olfactory tract; then it sends the striate arteries that run dorsally, following the medial border of the external capsule, to supply the lateral and dorsal portions of the caudate-putaminal complex. After originating the CoStA, the MCA bends over the lateral surface of the cerebral hemisphere, and sends branches in a variable manner, usually forming three groups: rostral, medial and caudal. In 65.51% of the cases the A1 segment was a single vessel on each side. However, in 34.48% of cases there was a duplication of this segment, near its entry into the longitudinal cerebral fissure. This duplicity produced a slit image of the "buttonhole" kind. Brown [6] described the duplicity of the A1 segment as being frequent, but did not quantify it. He also stated that there is no explanation to its meaning. It is remarkable that in 63.21% of cases the A1 segments showed a single anastomosis inside the longitudinal cerebral fissure, which was the junction of the two branches to form the azygos A2 segment. In the remaining 36.78% of cases, the A1 segments communicated two or more times before forming the A2 segment. These communications occurred at the point where the A1 segments penetrated the longitudinal cerebral fissure in 75% of cases, and in the depth of the fissure in 12.5% of cases. According to Greene [9], the ACAs can communicate near the longitudinal cerebral fissure by a simple plexus from which the A2 azygos segment emerges. Soares *et al*. [8] found a single anterior communicating artery in 32% of cases, and an anastomosis between the A1 segments in other 18% of cases, totaling 50% of cases with communications between the A1 segments. The results found in this study agree with those, and was found no communications like the one described by Greene [9]. According to Scremin [15], after the emergence of the NOA, the ACA originates the lateral orbitofrontal artery that irrigates the olfactory tubercle, the ventral surface of the olfactory bulb and rostral portion of the nucleus accumbens. Also, according to this author, the A2 azygos segment emits a medial orbitofrontal branch for each hemisphere, an upward septal branch and two to four small rostral septal branches, after which the A2 segment ascends in the dorsal and slightly caudal direction until it curls over the genu of the corpus callosum, continuing up from that point as the pericallosal artery. Regarding the place of origin of the ventral olfactory artery (VOA), the most common site was the A1 segment (83.90% of cases on the left and 82.75% on the right). In the rest of the cases (16.10% on the left and 17.25% on the right), the VOA originated from the A2 segment. Moffat [5] reported that the VOA rises from the ACA just before or just after the junction of the A1 segments. The observations of this study agree with those. Finally, regarding the place of junction of the A1 segments, it occurred while forming the azygos A2 segment of the ACA in 72.41% as a simple fusion of A1 segments. In the other cases (27.59%) it represented the continuation of the dominant A1 segment (70.83% on the left and 29.17% on the right). This continuation happened after a small lateral communication with its namesake. Moffat [5] noted that the two ACAs met in an acute angle, forming a single median dorsal vessel that ran dorsally and then caudally over the corpus callosum. Brown [6] found two types of formation of the A2 segment: the first one by fusing longitudinally side by side, resulting in a single vessel which enters the longitudinal cerebral fissure and then sends branches, and the second one by a short tangential fusion, enough to form an anastomosis, but not a distinct vessel. This discrepancy regarding the layout of the vessels that originate from the rostral part of the arterial circle should be attributed to different interpretations made by the observers at the time of establishing the outlines of the circles. This problem can be minimized by taking photographs of the dissected pieces.

CONCLUSIONS

The technique of vascular staining with latex allowed good visualization of the arteries studied, making the microdissection possible.

The constant patterns identified in all brains were as following:

1. The circle is a closed structure, all components are present, and anastomosis with each other;
2. MCA and NOA are always present;
3. The SCbAs always showed as terminal branches of the BA;
4. The A2 segment of the ACA is always an azygos vessel.

Among non-constant patterns, but often observed, highlighted the following:

1. The variation of the origin of PCAs ;
2. The presence of the TPAs, AChAs and CoAmAs;
3. The singleness of the origin of the MCA and the A1 segments of ACAs;
4. The presence of anastomosis between A1 segments of ACAs;
5. The origin of the VOA in the A1 segment of ACA;
6. The junction of A1 segments of ACA in simple fusion to form the azygos A2 segment.

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