Arteriolar Branching Patterns in the Brain of the Fruit Bats (*Pteropus lylei*)

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Abstract

The traditional view that Old World fruit bats (Megachiroptera) and insect or echolocating bats (Microchiroptera) are closely related has been challenged by claims that megachiroptera are the sister group to flying lemurs (Dermoptera) or Primates. Thus, the vascular morphology was examined to ascertain whether the arteriolar branching patterns in the head region of the fruit bat might reveal the primate characteristics. The arteriolar branching patterns for four regions of the brain (cerebrum, cerebellum, pons and medulla) in the fruit bat (*Pteropus lylei*) have been studied in detail using the vascular corrosion cast / SEM technique. It is found that most of the penetrating arterioles in all four regions of the brain run perpendicularly to their superficial artery. The medullar penetrating arterioles are tortuous after branching. The right angle branching in the cerebrum and cerebellum are similar in the primate, thus, this anatomical feature may be indicates the relative evolution between the megachiroptera and primate. Furthermore, this evidence may cause the reduction of the risk factor for atherosclerosis in the brain while decreasing the arterial blood pressure. The tortuous penetrating arterioles in the medulla may be suitable for the movement of the upper cervical joint. However, evidence that concerning the arteriolar branching patterns of the microchiroptera is not observed. In addition, further study in the angioarchitecture of the microchiroptera's brain in relation to megachiroptera and other primates is suggested.

Keywords: penetrating arterioles, tortuous arterioles, megachiroptera, primate, fruit bat

Introduction

The world's bats (order Chiroptera) can be readily separated into two suborders on the basis of morphological and behavioral characters: (*i*) suborder Megachiroptera, comprising of the single family Pteropodidae, and (*ii*) suborder Microchiroptera, encompassing all the other families. The megachiroptera includes the large fruit-eating bats being found only in the Old World, whereas the microchiroptera are primarily insectivorous occurring in the New World. Whether these two taxa represent a natural, monophyletic group, or whether the megachiroptera represent an early offshoot of the primate lineage, unrelated to the microchiroptera lineage, is unclear. Morphological features of the forelimbs related to flight suggest a sister relationship for the bats (Wible and Novacek, 1988). However, the megachiroptera has an advanced retinotectal pathway with a vertical hemidecussation of the kind previously found only in primates. In contrast, the microchiropteran bat has the "ancestral" or symplesiomorphous pattern of retinotectal connections so far found in all vertebrates except primates (Pettigrew, 1986). Kleinschmidt and co-worker (1986) suggested a large distance between microchiroptera and megachiroptera by studying the primary structures of the alpha- and beta-globin chains from heamoglobins.

The lylei's flying fox (*Pteropus lylei*) is one of the endangered species of Thailand and classified in order Chiroptera, suborder Megachiroptera. It is found along the coastal area of the Gulf of Thailand, south to Phetburi, north to Ayutthaya, and east to Combodia; also reported from Krabi Province and Saigon, Vietnam (Legakul and Mcneely, 1998). The detail description of the angioarchitecture in the lylei's flying fox's brain is not available.

Evidence of this issue will demonstrate the arteriolar branching pattern of the lylei's flying fox's brain and whether it was similar to the other primates.

The vascular corrosion cast technique in conjunction with the scanning electron microscope (SEM) was first described by Murakami (1971). He suggested the application of SEM in combination with the injection of plastic mixture to study fine distribution of blood vessels and delicate capillaries such as those of intestinal villi. During the years 1988 and 1989, this technique has been improved and widely used to obtain three-dimensional images with high resolution (Ritonga, 1988). Therefore, this technique is applied in this study.

Materials and methods

Fifteen adult fruit bats (*Pteropus lylei*) in both sexes, weighing between 250-260 g, were used. The animals were prepared for studying the arteriolar branching in the brain by the vascular corrosion cast technique in conjunction with scanning electron microscope.

Each animal was anaesthetized by chloroform inhalation before the thoracic cavity was cut. The 0.05 ml of heparin (Leo, 5000 IU/ml) was immediately injected into the left ventricle and allowed to circulate for about 1 minute. Then, a blunt needle was inserted into the lumen of the ascending aorta through the left ventricle. The needle was held in place with arterial clamps and subsequently, the right atrium was cut for draining the blood and injected fluid out. About 500-1,200 ml of 0.9% NaCl solution was perfused through the cannula to rinse the blood from the circulation. The blood and excessive injected fluid were running out through the right atrium, which was previously cut.

Immediately after 0.9% NaCl perfusion, each animal was manually injected with 50 ml of freshly prepared Batson's # 17 Plastic mixtures at the rate of 8-10 ml/min through the ascending aorta. The plastic mixture was completely filled into the blood vessels, until it was flowed out from the right atrium. The great vessels at the root of the heart were clamped to prevent the leakage of the plastic mixture. Each animal was left at room temperature for 1 to 2 hours for hardening and polymerizing of the plastic casts. The heads of the fruit bats were cut at the level of first cervical vertebrae, then skin and tissues were removed. The heads were placed in 10% formic acid for a couple days to decalcify bones. The brains were removed and divided into three regions; the cerebral hemispheres, cerebellum and the brainstem. Each region was cut in several directions; cross-sectioned, long-sectioned, midsagittal and parasagittal sectioned, and also cracked for exposing the internal aspect.

All of the specimens were placed in 40% KOH at room temperature for 15 days with daily changing of the solution. Then, the corroded specimens were rinsed in tap water and gentle washed with distilled water to remove the remaining tissues. The air-dried vascular casts were examined under stereomicroscope to select the areas for further investigation under SEM. The specimens were mounted on the metal stub, coated with gold/palladium. The vascular casts were examined and photographed under SEM at an accelerating voltage of 15 kV for study the arteriolar branching patterns in the brain.

Results

The penetrating arterioles of the brain are studied in four regions, including cerebrum, cerebellum, pons and medulla oblongata.

The superficial cerebral artery passing through the cerebral sulcus gives rise to the cerebral arterioles to supply the internal cerebral regions. Each of the cerebral arterioles often makes a right angle to the superficial at their junction (Figure 1). In addition, these arterioles branch into the capillary plexuses supplying the brain tissue.

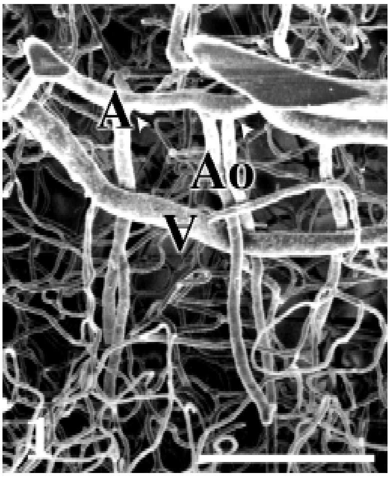


Figure 1. SEM micrograph of the cerebral vascular cast showing the perpendicularly branching (arrow head) of the cerebral arterioles (Ao). A, the superficial cerebral artery; V, the superficial cerebral vein. Bar = 176 µm.

The cerebellar arteries locate along the cerebellar cortex. They give rise to the penetrating arterioles, which supply the intracortical areas of the cerebellum. These cerebellar arterioles pass into the cerebellar sulcus of each cerebellar leaflet, and the direction of their penetrating often shows perpendicularly to cerebellar surface (Figure 2).

Figure 2. SEM micrograph of the cerebellar vascular cast showing the numerous perpendicularly branching (arrow head) of the cerebellar arterioles into the cerebellar leaf-let. Bar = 600 µm.

The pontine penetrating arterioles are also observed. They emerge into the internal part of pons, and make a right angle to the superficial pontine artery to supply the deep pontine structures (Figure 3).

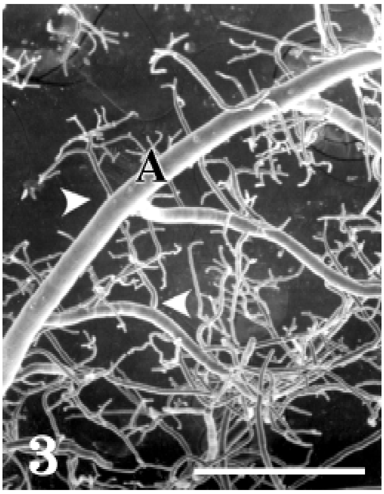
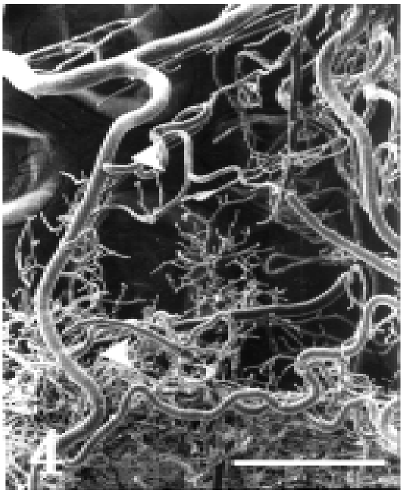


Figure 3. SEM micrograph of the pontine vascular cast showing the perpendicularly branching (arrow head) of the pontine arterioles. A, the superficial pontine artery. Bar = 500 µm.

The medullar penetrating arterioles are branched from the vertebral-basilar arteries to supply the internal structures of the medulla. The vertebral-basilar artery gives rise to its arterioles in a right angle (Figure 4). After branching, these arterioles become the tortuous pattern (Figure 5) before giving rise to the capillary plexuses in the medulla oblongata.



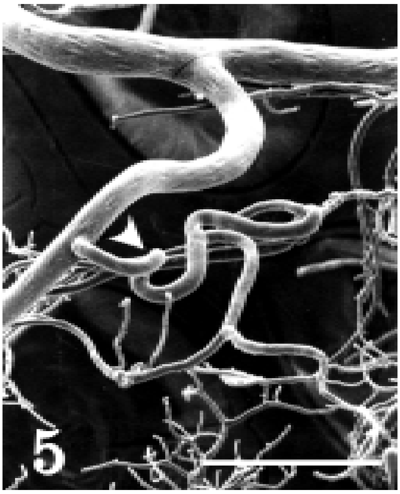


Figure 4. SEM micrograph of the medulla oblongata vascular cast showing the perpendicularly branching (arrow head) of the medullar arterioles. A, the superficial medullar artery. Bar = 380 µm.
Figure 5. SEM micrograph of the medulla oblongata vascular cast showing the tortuous medullar arteriole (arrow head). Bar = 200 µm.

Discussion

This paper demonstrates that the corrosion cast technique in conjunction with SEM is very useful for the study of the angioarchitecture. It is shown that the penetrating arterioles in the cerebrum and cerebellum are perpendiculary to the superficial artery. This finding is conformed to what has been reported in the primates, i.e. human cerebrum (Devnervoy *et al.*, 1981; Lametschwandtner *et al.*, 1990), human cerebellum (Devnervoy *et al.*, 1983), tree shrew cerebrum (Poonkhum *et al.*, 2000) and tree shrew cerebellum (Khomphatraporn, 1998). This finding indicates the morphological similarities between megachiroptera and primate. However, the perpendicular pattern of the pontine and medullar arterioles have not been documented. Hence, this is the first evidence about these configurations. Furthermore, some authors satisfied of placing megachiroptera in the order Primates by DNA sequence data from mitochondrial and nuclear gene (Mindell *et al.*, 1991). These findings are congruent with morphological characters including details of wing structure as well as cladistic analyses of amino acid sequences for three globin genes and indicate that neurological similarities between megachiroptera and primate are due to convergent evolution rather than to inheritance from a common ancestor.

Hademenos (1997) reported that the carotid-artery bifurcation is a common site of atherosclerotic disease because of 1) the acute angle between the external and internal carotid arteries and 2) the rapid flow at this junction. These two features could initiate the turbulent flow in the arterial dividing point. The right arteriole branching pattern in forementioned mammalian brains may reduce the risk factor for atherosclerosis in their brains.

The medulla is the most caudal part of the brain which continues to be the spinal cord. It is also located in a movable region of the neck. Thus, the tortuous pattern of the medullar penetrating arterioles may be useful for rupture prevention while there is movement at the upper cervical joint, especially atlanto-axial joint.

Though, the results of this study show the morphological similarities between megachiroptera and primate. The arteriolar branching patterns of the microchiroptera brain did not observed. Therefore, we suggest future research in this aspect.

Conclusions

By employing the vascular corrosion cast/SEM technique, the results have clearly shown the three-dimensional configuration of the arteriolar branching patterns in four areas of the fruit bat (*Pteropus lylei*)'s brain. The penetrating arterioles of cerebrum, cerebellum, pons and medulla oblongata run perpendicularly to their arterial surfaces. The medullar penetrating arterioles are branched in a tortuous pattern as emerging from the medullar artery. The perpendicular branching patterns in the cerebrum and cerebellum are similar to that has been reported in the primate. Thus, the megachiroptera and primate may have close evolution.

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