MICROVASCULARIZATION OF PONS IN THE COMMON TREE SHREW (*Tupaia glis*)

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Pons is the part of the brainstem connecting with the midbrain cranially and with the medulla oblongata caudally. It acts as a bridge between the right and left cerebellar hemispheres. It contains many important cranial nerve nuclei and tracts. Embryologically, mammalian pons is derived from the mesencephalic part of the hindbrain and it does not appear in reptiles or birds. The functions of pons relate with facial sensation, facial muscular control, chewing and lateral eye movement. Therefore, it is of interest to study the blood supply of pons, especially in the common tree shrew, which is regarded as a primitive primate.

The animals were divided into 3 groups. The first group was for gross observation, the second group was for the study of microvascularization using corrosion cast and scanning electron microscopy technique and the third group was for histological study using light microscope. It was found that the area of blood supply at the caudal and rostral pons could be divided into anteromedial, anterolateral, lateral and posterior areas. The main blood supply of pons come from the basilar artery and its branches. These are medial, pontine, anterior inferior cerebellar and superior cerebellar arteries. The main blood supply of the caudal pons is from branches of anterior inferior cerebellar artery, while the rostral pons is from the superior cerebellar artery.

The significance of this study is the blood supply of the cranial nerve nuclei, which originates from pons, is clearly demonstrated and the pattern of the vascular supply is shown and discussed. The venous drainage of caudal pons is drained into the anterior pontomesencephalic and the petrosal veins. The venous drainage of the rostral pons flows into a vein of the pontomesencephalic sulcus and precentral cerebellar veins.

The results of this study show also that the common tree shrew should be classified as a higher mammal. These results suggest future research can use the common tree shrew as an experimental model to further knowledge and research about the structures, patterns and functions of pons.

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อิทธิพล พวงเพชร : การศึกษาโครงสร้างและการกระจายของหลอดเลือดที่มาเลี้ยงก้านสมองส่วนพอนส์ของกระแต [MICROVASCULARIZATION OF PONS IN THE COMMON TREE SHREW (*Tupaia glis*)]. คณะกรรมการควบคุมวิทยานิพนธ์ : เรือน สมณะ, พบ., Ph.D., วิสุทธิ์ ประดิษฐ์อาชีพ, ปร.ด., วันทนีย์ ตระกูลรังสิ, วท.ม. 82 หน้า ISBN 974-664-495-5

พอนส์เป็นส่วนหนึ่งของก้านสมอง ทางด้านบนติดต่อกับ midbrain ด้านล่างต่อกับ medulla oblongata เป็นสมองส่วนที่เชื่อมต่อซีรีเบลลัมทั้งสองข้างเข้าด้วยกัน ภายในมีกลุ่มนิวเคลียสและทางเดินประสาทที่สำคัญมากมาย พอนส์มีหน้าที่เกี่ยวกับการรับความรู้สึกของหน้า การแสดงสีหน้า การเคี้ยว และการเหลือบตามองด้านข้าง การศึกษานี้เกี่ยวกับหลอดเลือดที่มาเลี้ยงพอนส์ โดยใช้กระแตซึ่งเป็นสัตว์ที่อยู่ในลำดับเดียวกับมนุษย์เพื่อการศึกษา โดยแบ่งกระแตออกเป็น 3 กลุ่ม กลุ่มแรกสำหรับศึกษาโครงสร้างภายนอก กลุ่มที่สองสำหรับศึกษาโครงสร้างและการกระจายของหลอดเลือดโดยใช้เทคนิค vascular corrosion cast ร่วมกับการใช้กล้องจุลทรรศน์แบบส่องกราด (SEM) กลุ่มที่สามสำหรับศึกษากลุ่มนิวเคลียสและทางเดินประสาท โดยใช้กล้องจุลทรรศน์แบบธรรมดา (LM) จากการศึกษาพบว่าหลอดเลือดหลักที่มาเลี้ยงพอนส์มาจาก basilar artery และแขนงของมันซึ่งประกอบด้วยหลอดเลือดแดง superior cerebellar หลอดเลือดแดง anterior inferior cerebellar และหลอดเลือดแดง pontine arteries โดยสามารถแบ่งบริเวณที่เลี้ยงโดยหลอดเลือดต่างๆ ตามจุดที่หลอดเลือดแทงเข้าไปและตำแหน่งที่หลอดเลือดนั้นมาเลี้ยง ได้เป็น 4 บริเวณ คือ บริเวณแนวกลาง บริเวณด้านข้างแนวกลาง บริเวณด้านข้าง และ บริเวณด้านหลัง จากการศึกษาพบว่าบริเวณแนวกลางได้รับเลือดมาจาก medial pontine artery และ paramedial artery ซึ่งเป็นแขนงของ basilar artery บริเวณแนวกลางด้านข้างและบริเวณของพอนส์ด้านข้างได้รับเลือดจาก rostral medulla artery, inferior pontine artery, middle pontine artery, rostral pontine artery และ anterior inferior cerebellar artery บริเวณด้านหลังส่วนกลางและส่วนล่างได้รับเลือดจาก anterior inferior cerebellar artery บริเวณส่วนบนได้รับเลือดจากแขนงของ superior cerebellar artery และ rostral pontine artery เป็นที่น่าสังเกตว่า trigeminal nucleus ได้รับเลือดจาก anterior inferior cerebellar artery, middle pontine artery และ rostral pontine artery แล้วเลือดดำจะไหลเข้าสู่ petrosal vein ส่วน abducent nucleus ได้รับเลือดจาก medial pontine artery, และ anterior inferior cerebellar artery แล้วเลือดดำจะไหลเข้า medial pontine vein และ petrosal vein ส่วน facial nucleus ได้รับเลือดจาก anterior inferior cerebellar artery และเลือดดำจะไหลเข้า petrosal vein ส่วน vestibular และ cochlear nucleus ได้รับเลือดจาก anterior inferior cerebellar artery แล้วเลือดจะไหลเข้า petrosal vein เป็นที่น่าสังเกตว่าเลือดดำจากบริเวณแนวกลางจะเทเข้าสู่ anterior pontomesencephalic vein และ vein of pontomesencephalic sulcus เลือดดำจากบริเวณแนวกลางด้านข้างและด้านข้างของพอนส์จะเทเข้าสู่ petrosal vein และ anterolateral pontomesencephalic vein ส่วนเลือดดำจากบริเวณด้านหลังจะไหลเข้าสู่ precentral cerebellar vein, petrosal vein และ anterior pontomesencephalic vein.

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LIST OF ABBREVIATIONS

AA	=	Anterior subdivision of anterior division of anteroventral cochlear nucleus
Abd	=	Abducent nucleus
AChAHB	=	Anterior choroidal artery of hindbrain
AICA	=	Anterior inferior cerebellar artery
ALPMV	=	Anterolateral pontomesencephalic vein
AMV	=	Anterior medullary vein
AP	=	Posterior subdivision of anterior division of anteroventral cochlear nucleus
APMV	=	Anterior pontomesencephalic vein
BA	=	Basilar artery
BV	=	Basilar vein
CAE	=	Locus ceruleus
Cbc	=	Cerebral cortex
Cbl	=	Cerebellum
CN	=	Cochlear nucleus
DTF	=	Dorsal tegmental field
CPA	=	Caudal pontine artery
FN	=	Facial nucleus
IC	=	Inferior colliculus
ICP	=	Inferior cerebellar peduncle

LIST OF ABBREVIATIONS (CONT)

ID	=	Inferior division of anterior inferior cerebellar artery
IPA	=	Inferior pontine artery
IPS	=	Inferior petrosal sinus
LD	=	Lateral division of superior cerebellar artery
LSO	=	Lateral superior olivary nucleus
LTF	=	Lateral tegmental field
M	=	Medulla oblongata
MB	=	Midbrain
MCP	=	Middle cerebellar peduncle
MD	=	Medial division of superior cerebellar artery
ML	=	Medial lemniscus
MLB	=	Medial longitudinal bundle
MPA	=	Middle pontine artery
MPB	=	Medial parabrachial nucleus
MSO	=	Medial superior olivary nucleus
MTF	=	Medial tegmental field
NTB	=	Nucleus of trapezoid body
OB	=	Olfactory bulb

LIST OF ABBREVIATIONS (CONT)

OL	=	Olfactory lobe
PA	=	Paramedial artery
PAD	=	Posterodorsal subdivision of the anterior division of the anteroventral cochlear nucleus
PcV	=	Precentral cerebellar vein
PT	=	Pyramidal tract
PV	=	Petrosal vein
RD	=	Rostral division of superior cerebellar artery
RMA	=	Rostral medulla artery
RPA	=	Rostral pontine artery
RS	=	Rectus sinus
SCA	=	Superior cerebellar artery
SCP	=	Superior cerebellar peduncle
SD	=	Superior division of anterior inferior cerebellar artery
SO	=	Superior olivary nucleus
SS	=	Sigmoid sinus
SSS	=	Superior sagittal sinus
TA	=	Trigeminal artery

LIST OF ABBREVIATIONS (CONT)

TB	=	Trapezoid body
TPV	=	Transverse pontine vein
TS	=	Transverse sinus
VA	=	Vertebral artery
VIN	=	Inferior vestibular nucleus
VMN	=	Medial vestibular nucleus
VPMS	=	Vein of pontomesencephalic sulcus
5M	=	Motor trigeminal nucleus
5P	=	Principle sensory of the trigeminal nucleus
5S	=	Spinal trigeminal nucleus
5T	=	Spinal trigeminal tract

CHAPTER I

INTRODUCTION

The pons is a portion of the hindbrain on which the cerebellum develops. It connects with the midbrain cranially and with the medulla oblongata caudally (1). It acts as a bridge placing between the right and left cerebellar hemispheres (2). The caudal limit of the pons is marked by the plane passing through the striae medullares pontis dorsally through the caudal end of the pontine fibers ventrally. The rostral limit of the pons is plane just posterior to the inferior colliculus dorsally and to the cerebral peduncles ventrally (3). Embryologically, the pons is derived from metencephalic part of the hindbrain (4). Phylogenically, it presents in the mammalian brains but it is not observed in the reptiles or birds. Its size varies with the development of cerebral hemispheres (2). The pons consists of the dorsal or tegmental portion and the ventral or basilar portion. They differ greatly in structures (5). The large ventral part composes of (a) longitudinal descending fiber bundles, (b) pontine nuclei and (c) transversely oriented fibers projecting into the cerebellum. A smaller dorsal part or tegmentum contains aggregations of cells and fibers that form a central core known as the reticular formation. The reticular formation in the pontine tegmentum continue from the reticular formation of the medulla and midbrain. Cranial nerve nuclei, ascending sensory systems as well as descending motor pathways arising from brainstem nuclei are found within tegmentum. Cranial nerve nuclei that associate with the pons are the trigeminal (CN V), abducent (CN VI), facial

(CN VII), and the two components of the vestibulocochlear (CN VIII) nerves. The abducent nucleus lies in the floor of the fourth ventricle and is partially encircled by fibers of the facial nerve. The facial nerve fibers and cells in the abducent nucleus underline the facial colliculus in the floor of the fourth ventricle. Fibers of the abducent nerve emerge from the ventral surface of the brainstem at the junction of the pons and medulla. The facial and vestibulocochlear nerves emerge and enter the lateral surface of the pons at the cerebellopontine angle, junction among the pons, medulla and cerebellum. The trigeminal nerve consists of motor and sensory fibers traverse rostrolateral part of the middle cerebellar peduncle to reach nuclei in the dorsolateral pontine tegmentum. The middle cerebellar peduncle, with massive collections of cross fibers arising from pontine nuclei, projects to the opposite cerebellar hemisphere (6).

The functions of pons concern with feeding, emotional expression (trigeminal, salivary, and facial nuclei), lateral viewing and equilibrium. Pons is the higher center for respiratory control (3). The inspiration is evoked from the tegmentum through the site of the pneumotaxic center (7). Tang (8) found that the pneumotaxic center in the cat lay in the dorsolateral portion of the pontine tegmentum. Wang (9) believed that the nucleus parabrachialis medialis in pons are also the pneumotaxic center. In addition, pons is the important relay station in the pathway between cerebral cortex and cerebellum (10). Furthermore, lesions of the pons due to basilar artery branch occlusion (11) and pontine hemorrhage (12, 13) could lead to locked-in syndrome in the patients (14, 15, 16, 17). It is evident that most of the pons lesions are due to blood supply impairment (11, 12, 13).

The study using angiography reveals that the pons is supplied by vertebrobasilar system (18, 19). In human, the pons is supplied by the basilar artery, the anterior inferior and superior cerebellar arteries (1). Angiographic study in the guinea pig indicates that the blood supply of pons comes from the vertebral and basilar arteries (20). There are three groups of branches arising from these arteries, a medial (anterior), lateral, and posterior groups. The medial group arises from basilar artery piercing the pons along the medial plane and supplies the pyramid, reticular formation, nucleus of hypoglossal nerve and medial part of central gray. The lateral group arises from the anterior inferior cerebellar and basilar arteries supplying the lateral and dorsal parts of pons. The posterior group originates from the posterior inferior cerebellar and posterior spinal arteries. This group supplies the inferior part of the floor of fourth ventricle and inferior cerebellar peduncle and terminating at the level of gracile and cuneate nuclei. Gabrielsen and Amundsen (21) found that pontine branches of the basilar artery could be divided into the medial and the transverse (circumferential) branches. The medial branches arise from posterior aspects of basilar artery and enter the anterior part of the pons along the median groove. The transverse branches are variable in size and number. They originate bilaterally from the posterolateral aspects of the basilar artery. In addition, Hassler (22) found that the medial branches are divided into medial and paramedial arteries, the medial artery in the upper part of the pons come caudalward. All paramedial arteries run medially toward the floor of the fourth ventricle. The tegmentum of the pons receives blood supply from short and long circumferential and paramedian arteries (22). In the dog the posterior inferior cerebellar arteries arise from basilar artery. The basilar artery in the dog is very long. It lies on the ventral surface of both pons and medulla. Furthermore, the posterior cerebral arteries and superior cerebellar arteries are branches of posterior communicating arteries in the dog (23, 24). The venous blood of the pons is drained in anterior direction into the anterior pontomesencephalic vein (25). That from the lateral pontine tegmentum is mainly drained into the lateral mesencephalic vein (26). The blood in the pontomesencephalic vein drains primary into (a) the superior petrosal vein mostly through the petrosal vein and (b) peduncular vein and further into the carvernous sinus (27).

Michalska (28) has studied the blood supply of the pons in guinea pig by injection the microfil silicone resin into blood vessels and found that the pons is supplied by 3 groups of blood vesseles, namely, medial (anterior), lateral and posterior groups. The medial group is further subdivided into anteromedial and anterolateral groups. The anteromedial group comprises of pontine branches of the basilar artery. These branches consist of the long vessels reaching medial longitudinal fasciculus. They also give off twigs supplying fibers of corticocerebellar tracts, and the medial lemniscus. The venous blood from this area is drained into basilar vein. The anterolateral group is formed by branches of basilar and anterior inferior cerebellar arteries. This group supplies medial lemniscus and gives off rami to the corticopontine and pontocerebellar tracts. The venous blood from this area is drained into basilar vein. The lateral group arises from the basilar and anterior inferior cerebellar arteries supplying lateral and dorsal parts of pons. The venous blood from this area empties into basilar vein. The posterior group is formed mainly by branches of the superior cerebellar artery and supplies the superior part of the pons, fourth ventricle, lateral lemniscus, central tegmental tract, and the superior cerebellar peduncle. From the posterior group, the highest density of vascular network is in the superior olivary, abducent, and sensory trigeminal nuclei. Mean density of vessels is in the motor trigeminal nucleus. The lowest density is in the corticospinal and corticothalamic tracts. The venous blood from this area is drained into the superior cerebellar veins.

The methylmethacrylate plastic mixture has been shown by Batson (29) to be with very good property of filling the cavities of hollow organs including capillaries. Murakami (30) injected the plastic mixture in combination with the application of the scanning electron microscope. He could clearly demonstrate the distributions of blood vessels including delicate capillaries in three dimension. In the past decade, this technique (vascular corrosion cast technique) has been employed to demonstrate the vascular pattern in various organs of the common tree shrew (31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47). Eventhough, the morphological features and vascular configuration of pons have been demonstrated in human (21, 22), dog (23, 24) and guinea pig (20, 28) but with other techniques. Furthermore, detail information concerning the angioarchitectures of this organ is still limit, especially that of the primate. Therefore, it is of interest to elucidate the microanatomy and angioarchitecture of pons in the common tree shrew (*Tupaia glis*), the animal regarded as a primitive primate (48, 49, 50).

CHAPTER II

OBJECTIVES

The aims of the present study are :

1. To illustrate gross anatomy and microanatomy of pons in the common tree shrew (*Tupaia glis)*

2. To elucidate the angioarchitecture in various pontine structures

3. To compare this findings with those of other species

CHAPTER III

MATERIALS AND METHODS

Animal Preparation

Twenty adults common tree shrews of both sexes weighing between 110 to 170 gm were used for the study of pons. The animals were divided into three groups. The first group of four animals was for gross observation by dissection of their brains. Eight animals in the second group were injected with the Batson's # 17 plastic mixture to examine the vascular bed in pons under stereomicroscope and SEM. The third group of eight animals was processed for light microscopic (LM) study. Under diethyl ether anesthesia the thoracic cage of each animal was opened, the 0.05 ml heparin (Leo, 5,000 IU/ml) was immediately injected into the left ventricle and allow to circulate for 1 to 2 minutes. A blunt needle (# 18 gauge) was cannulated into the ascending aorta through the left ventricle and securely hold in place with an arterial clamp. Thereafter, the right atrium was opened to serve as efferent port for the out flowing blood. The 0.9 % NaCl solution (about 150 to 200 ml) was flushed through the cannula to get rid of the blood from the circulation since the remaining blood cells could be obstacles to the injection of the 10 % formalin or plastic mixture.

For Gross Observation (Group I)

After the washing the blood with 0.9% NaCl solution the 10 % formalin were injected manually via the same needle into ascending aorta to fix the tissue. The head of the animals were cut and fixed in the same fixative for 3 days. After rinsing several times with tap water the brain were removed from the skull (Fig. 1, 2). Under stereomicroscope, the brains were dissected to expose the external morphology of the pons.

For Vascular Corrosion Cast /SEM (Group II)

Immediately, after 0.9 % NaCl solution (about 150 to 200 ml) perfusion, each animal was injected with approximately 23 ml of the freshly prepared Batson's # 17 plastic mixture according to the method described by Chunhabundit and Somana (51) at the optimum rate of 8 ml/min (52). Most of vascular beds would be completely filled with the plastic mixture when it flowed out from right atrium. The root of the heart was clamped to prevent leakage of the unpolymerized plastic. The animals were placed at room temperature for 30 minutes to let the casting medium hardening. The heads of animal were removed and immersed in hot water (60 $^{\circ}$C to 80 $^{\circ}$C) for 2 h to accelerate the polymerization of the plastic cast. The skin and tissue around the skull was removed before being macerated in 40 % KOH solution and immersed in water bath at temperature 60 $^{\circ}$C for 3 to 4 days with changing of KOH solution daily. Each specimen was rinsed in a slow running tap water and gently washes several times with distilled water to remove the remaining tissues. The vascular casts were filled with distilled water and placed in the deep freezer at the temperature of –70 °C for 45 min. Thereafter, the frozen vascular casts were placed in lyophilizer until dry. Under stereomicroscope, the vascular casts were inspected and microdissected to identify the main arterial supply and venous drainage of the pons. The dry vascular casts of pons were mounted onto the metal stubs, coated with gold/palladium at 60 nm thick in ion sputtering apparatus, examined and photographed under a SEM at an acceleration voltage of 15 kV.

For Light Microscopy (Group III)

Immediately after 0.9 % NaCl perfusion, 250 ml of Bouin's solution were injected manually through the ascending aorta to fix the tissue. The brains were dissected and fixed in the same fixative overnight. After rinsing several times with tap water, the specimens were dehydrated in a graded series of ethanol, infiltrated and embedded in paraffin. The pons were serial sectioned at 5 to 7 μm thick, stained with cresyl violet to identify the groups of nuclei. The serial sections were viewed and photographed under light microscope.

CHAPTER IV

RESULTS

Pons is the part of brainstem situating between the midbrain superiorly and medulla oblongata inferiorly. Its dorsal portion connects with cerebellar hemisphere (Fig. 5). In the tree shrew, it is lying on the clivus part of the sphenoid bone in the posterior cranial fossa. The pons is approximately 4 mm wide, 7 mm long and 5 mm dorsoventrally.

Its caudal limit of the pons is marked by the plane passing through the lower border of middle cerebellar peduncles dorsally (Fig. 4) and that through the originating of the AICA ventrally (Fig. 3). The rostral limit of pons is limited by the plane just posterior to inferior colliculus dorsally (Fig. 4) and to the cerebral peduncle ventrally (Fig. 3). The cranial nerves that originate from the pons are trigeminal, abducent, facial and vestibulocholear nerves (Fig. 3). The study of the sagittal sections reveals that its ventral part is larger than the dorsal part (Fig. 5). From this study, it is noted that the origin of abducent nerve and the origin of the AICA separate the lower border of ventral of pons from the medulla oblongata. These landmarks correspond with the caudal limit of the superior olivary nucleus revealed histologically.

The blood supply of pons in the common tree shrew is from the branches of the basilar artery (Fig. 6). At the rostral part of medulla oblongata, the left and right vertebral arteries join together to be the basilar artery. The basilar artery extends from the rostral part of the medulla oblongata to the rostral part of the pons before bifurcating and giving rise to the left and right branches. Each of these branches divides into the superior cerebellar and posterior cerebral arteries in the interpeduncular fossa (Fig. 6). The basilar artery and its branches namely, rostral medulla, pontine, anterior inferior cerebellar, superior cerebellar arteries are main sources of the arterial supply to the pons (Figs. 6, 7, 8). These branches of the basilar artery could be divided into circumferential and medial types.

The circumferential type consists mostly of branches from posterolateral aspect of the basilar artery. They lie at the surface of pons and run to lateral and posterior surfaces of the pons. These arteries could be identified from caudal pons to the rostral pons as rostral medulla arteries, anterior inferior cerebellar arteries, inferior pontine arteries, middle pontine arteries, trigeminal arteries, rostral pontine arteries and superior cerebellar arteries.

1. The rostral medulla artery (RMA) is a small branch at the upper part of medulla oblongata. It originates from lateral sides of basilar artery and runs to the lateral aspect of medulla oblongata and gives rostral and caudal branches. The rostral branches give off branches to supply the most caudal part of pons (Fig. 7).

2. The anterior inferior cerebellar artery (AICA) is large and important branch at the caudal part of pons. It is from lateral sides of basilar artery and runs to the dorsal aspect of pons and to the inferior part of the cerebellum. There are short branches from the proximal one third of AICA distributing blood to the superficial aspect of ventral part of pons. From proximal two thirds of AICA, there are long branches penetrating deep into the pons. Finally, each AICA bifurcates into superior and inferior divisions (Figs. 7, 10, 11).

2.1 The superior division (SD) gives off medial and lateral branches placing the pons in the horizontal plane. The medial branches give off short small arteries to supply the superior olivary nucleus and longer arteries penetrating the pons to supply the deep structures. The lateral branches run laterally and further divide to supply pontine nuclei (Figs. 6, 7).

2.2 The inferior division (ID) courses inferolaterally and gives off posterior branches to supply pontine nuclei. The other branches of inferior division are choroidal branches or anterior choroidal arteries of hindbrain (AChAHB). They supply choroid plexus of the fourth ventricle (Fig. 7). The labyrinthine artery is a branch of the inferior division of AICA. It supplies inner ear.

3. The inferior pontine artery (IPA) is derived from the middle portion of the basilar artery it runs along the posterolateral aspect of pons. This branch is short and further breaks into short and long branches to supply anterolateral area of pons (Fig. 8).

4. The middle pontine artery (MPA) gives off short and long branches to supply anterolateral and lateral areas of pons. These branches also penetrate into the pons to supply the trigeminal nerve and its nuclei (Fig. 8).

5. The trigeminal artery (TA) is from the upper one fourth of the basilar artery. It runs to the lateral part of pons and gives off the superior branches to supply the trigeminal ganglion (TG) and the inferior branches to supply the trigeminal nerve and middle cerebellar peduncle. It also gives off short branches to supply anterolateral area of pons (Fig. 8).

6. The rostral pontine artery (RPA) originates from the basilar artery just before basilar artery bifurcates. It curves laterally on the surface of the pons. At the begining of this artery, it gives off short branches that penetrate and supply the anteromedial and anterolateral areas of the rostral pons (Figs. 6, 8, 23).

7. The superior cerebellar artery (SCA) branches from the bifurcation of basilar artery. It is noted that the symmetrical appearance of SCA could be observed. This artery breaks into the rostral, lateral and medial divisions. The SCA gives off medial branches and intermediate branches to supply the anteromedial, anterolateral and lateral areas of rostral pons. The rostral division of SCA gives off branches to supply the posterior area of rostral pons (Figs. 6, 8, 20, 21).

The observation of the horizontal sections of pons with the light microscope after staining with cresyl violet reveals that the neurons are dark while fibers tracts are pales.

In the caudal sections of pons above the anterior inferior cerebellar arteries (AICA), the basis pontis is small but the tegmentum is large. The caudal area of pons could be divided into anteromedial, anterolateral, lateral and posterior areas in relation to the distribution of branches of the basilar artery (Fig. 9).

The anteromedial area contains the small pyramidal tract (PT) at ventromedial part of pons. The trapezoid body (TB) and medial lemniscus (ML) are the large group of fibers that run across pons dorsally to the pyramidal tract. The medial tegmental field (MTF) is located dorsally to the trapezoid body. The medial longitudinal bundle (MLB) is seen as a long pale area in the dorsomedial part. The ascending tract originated from facial nucleus is large and round situating in the most dorsomedial part. Lateral to the ascending tract, there are the abducent nucleus and its fibers running from dorsal to ventral surface (Fig. 9).

The anterolateral area contains of the large superior olivary nucleus (SO) which could be divided into medial (MSO) and lateral olivary nucleus (LSO). They situate on the lateral side of the abducent nerve fibers. The facial nucleus is seen just dorsal to the superior olivary nucleus (Fig. 9) .

The lateral area of the pons composes of the spinal trigeminal nucleus locating dorsally to its tract. The lateral tegmental field (LTF) is situating medial to the spinal trigeminal nucleus. The fibers from the facial nucleus could be observed between trigeminal nucleus and superior olivary nucleus. The inferior cerebellar peduncle (ICP) is located just lateral to the spinal trigeminal nucleus while the cochlear nucleus is in the most lateral area. The abducent nucleus is also seen just dorsal of this area (Fig. 9).

The posterior area contains the medial (VMN) and inferior vestibular nuclei (VIN) (Fig. 9).

The study with vascular corrosion cast technique of the horizontal section of caudal pons illustrates that the medial arteries originate from posterolateral aspect of basilar artery and run backward caudally to the floor of fourth ventricle. They give off branches which curve ventrally from the proximal one third in the ipsilateral side and a few branches to the contralateral side. Some branches are seen in horizontal plane (Figs. 10, 11, 11b, 26). The distal end of these arteries courses to the same side and reach the floor of the fourth ventricle and breaks into capillaries which anatomose to capillaries of other arteries. These arteries give blood to supply TB, MTF, ML, MLB, abducent nucleus and fibers of abducent nucleus in the anteromedial area. The anterolateral area receives the blood from caudal pontine arteries to supply fibers of abducent nucleus, superior olivary nucleus, MTF, and pyramidal tract (Fig. 11, 11a). The lateral area receives the blood from long branches of AICA to supply LTF, superior olivary, facial, spinal trigeminal and abducent nuclei (Figs. 10, 11). There are many small branches that penetrate the spinal trigeminal fibers and supply its lateral area with low density of blood vessels (Fig. 10). The other branches supply the cochlear nucleus. The vestibular nuclei in the posterior area are supplied by caudal pontine and branches of AICA (Figs. 10, 12).

As in the caudal pons, the rostral area could also be divided into four areas as anteromedial, anterolateral, lateral and posterior areas (Fig. 13).

In the anteromedial area, the pyramidal tract is located ventrally and anteriorly to the trapezoid body (TB) and medial lemniscus (ML) while the lateral tegmental field (LTF) is located dorsoventrally to the trapezoid body. The medial longitudinal bundle (MLB) is placing tranversely and ventrally to the dorsal tegmental field (DTF) (Fig. 13).

In the anterolateral area, the superior olivary nucleus could be divided into medial nucleus of superior olive (MSO) and lateral nucleus of superior olive (LSO). This nucleus is found ventral to the lateral tegmental field (LTF) (Fig. 13).

In the lateral area, the principal sensory trigeminal nucleus (5P) is located laterally to the motor trigeminal nucleus (5M). The middle cerebellar peduncle (MCP) connects to pons at the area just lateral to the principal sensory trigeminal nucleus (Fig. 13) .

In the posterior area, the locus ceruleus (CAE) is located laterally in adjacent to the dorsal tegmental field. The medial parabrachial nucleus (MPB) is located in the area lateral to the CAE. The superior cerebellar pedulcle (SCP) could be observed at posterolaterally to the dorsal tegmental field (DTF).

The study with vascular corrosion cast technique of the horizontal sections of the rostral pons reveals that there are a lot of medial arteries originating from the bifurcation of the basilar artery. These arteries supply pyramidal tract, trapezoid body, medial lemniscus and DTF of the anteromedial area (Figs. 14, 15, 16, 17). The SCA gives off small branches to supply the medial and lateral parts of the superior olivary nucleus, LTF, motor and principal sensory trigeminal nuclei and trigeminal tract in the anterolateral and lateral areas (Figs. 18, 19). The rostral division of SCA gives off branches to supply CAE and the superior cerebellar peduncle (SCP) of the posterior area (Figs. 14, 18).

The anteromedial area receives blood from the medial branches. The medial branches could be divided into medial and paramedial arteries.

1. The medial arteries (MA) are direct branches from the basilar artery. They originate just below the AICA, above the AICA, above trigeminal arteries, and at bifurcation of basilar artery. They supply the anteromedian area of pons (Figs. 17, 20, 25, 26).

2. The paramedial arteries (PA) are branches from the proximal part of the of pontine arteries, AICA and SCA. These arteries are not the direct branches from basilar artery. They supply the anteromedial area of pons (Figs. 17, 21).

The study of vascular cast under SEM reveals that the pons of the common tree shrew receives main blood supply from basilar artery and its branches. There are RMA, AICA, IPA, MPA, TA, RPA and SCA (Figs. 22, 23, 24, 25). They originate from posterolateral aspect of basilar artery then run to the lateral border of pons and break into short branches and long branches to supply deep tissues in the pons (Figs. 22, 23, 24, 25). It is noted that the arterial sphincters are often seen at the branching sites of the small arteries (Fig. 23). The branches that originate from the pontine artery are tortuous before breaking into arterioles and capillaries (Figs. 23, 24, 27. 28). The number of medial and paramedial arteries are less than the medial and paramedial veins (Fig. 25). At the lateral surface of pons, the small branches of AICA penetrate through the trigeminal nucleus and its tract before breaking into arterioles and capillaries to supply them (Figs. 28, 33). At the surface of vascular cast, the smooth muscle cells could be seen wrapping around the small arteries (Fig. 28). At the anteromedial area, the medial artery penetrates into the medial groove and supplies this area. Some branches courses ventrally before breaking into arterioles and capillaries (Figs. 26, 29). It is observed that the capillary network in the areas representing pontine nuclei are with higher density of capillaries than in the area containing nerve fibers (Fig. 31). When the surface of capillary cast is observed at high magnification, it appears rather smooth indicating that the capillaries in the pons are without fenestration (Fig. 30).

The venous blood of the pons is collected from the capillaries into small venules and further into the tributaries of veins before being emptied into the large veins in the ventral and lateral surface of pons (Figs. 32, 33, 34).

The deep group of veins of the pons collects the venous blood from the deep part of the pons. The venous blood from anterolmedial area is collected by medial veins before draining into the anterior pontomesencephalic vein (APMV) (Figs. 33, 34, 38). The venous blood from anterolateral area is collected by tributaries of anterolateral pontomessencephalic vein which drains into anterolateral pontomessencephalic vein (ALPMV) and further to the vein of pontomesencephalic sulcus (VPMS) (Figs. 26, 36). The venous blood from lateral and posterior areas is collected by tributaries of petrosal vein to drain into the petrosal vein (PV) (Figs. 7, 33). The venous blood from rostrodorsal is collected by tributaries of precentral cerebellar vein before draining into the precentral cerebellar vein (PcV) (Fig. 34).

The venous blood from the superficial areas of the pons could be collected into superficial veins as below :

1. The venous blood from ventromedial, ventrolateral surfaces of pons is collected into the anterior pontomesencephalic vein (APMV) (Figs. 35, 36). At the caudal pons, the anterior pontomesencephalic vein joins with petrosal vein (PV) laterally and anterior medullary vein (AMV) caudally (Fig. 7). At the middle part of pons, it deviates and joins with the transverse pontine vein (TPV) and further to anterolateral pontomesencephalic vein (ALPMV) (Figs. 35, 36) and basilar vein (BV). At the caudal end, it connects to anterior medullary vein (Fig. 7).

2. The venous blood from ventral surface of the middle part of the pons is drained into transverse pontine vein and further into the vein of pontomesencephalic sulcus (VPMS) (Fig. 36) and basilar vein (BV).

3. The venous blood from anterolateral surface of pons is drained into anterolateral pontomesencephalic vein (ALPMV) then joins with transverse pontine vein (TPV) before joining the vein of pontomesencephalic sulcus (VPMS) (Fig. 36)

4. The venous blood from lateral and dorsal surface is emptied into the petrosal vein (PV) which is located on the lateral side of pons. The petrosal vein drains into inferior petrosal sinus (IPS) and finally into sigmoid sinus (SS) (Figs. 7, 33).

5. The venous blood from rostral, ventral, lateral and dorsal portions of pons is drained by vein of pontomesencephalic sulcus which lies under the superior cerebellar artery at pontomesencephalic sulcus (Figs. 17, 21). It collects the venous blood from anterolateral pontomesencephalic vein to empty into the junction between transverse sinus (TS) and sigmoid sinus (SS) (Fig. 38).

6. The venous blood from rostrodorsal part of pons flows into precentral cerebellar vein (PcV)and further into the rectus sinus (RS) (Fig. 34).

Figure 1. Photograph of the common tree shrew brain, ventral view, after the animal had been injected with red plastic mixture. M, medulla oblongata; OB, olfactory bulb; Ol, olfactory lobe; PONS, pons. 3.5X

Figure 2. Photograph of dorsal view of the common tree shrew brain. Cbc, cerebral cortex; Cbl, cerebellum; asterisk, superior sagittal sinus; TS, transverse sinus. 3.5X

Figure 3. Photograph of the pons, ventral view, showing the basilar artery (BA), vertebral artery (VA) and cranial nerves. Arrowhead, trigeminal nerve; open arrowhead, abducent nerve; double open arrowheads, facial nerve; double arrowheads, vestibulocochlear nerve; asterisk, anterior inferior cerebellar artery. 8X

Figure 4. Photograph of the pons, dorsal view, showing the middle cerebellar peduncle (MCP) and distal end of AICA (arrowhead). Asterisk, tributaries of petrosal vein; IC, inferior colliculus. 8.5X

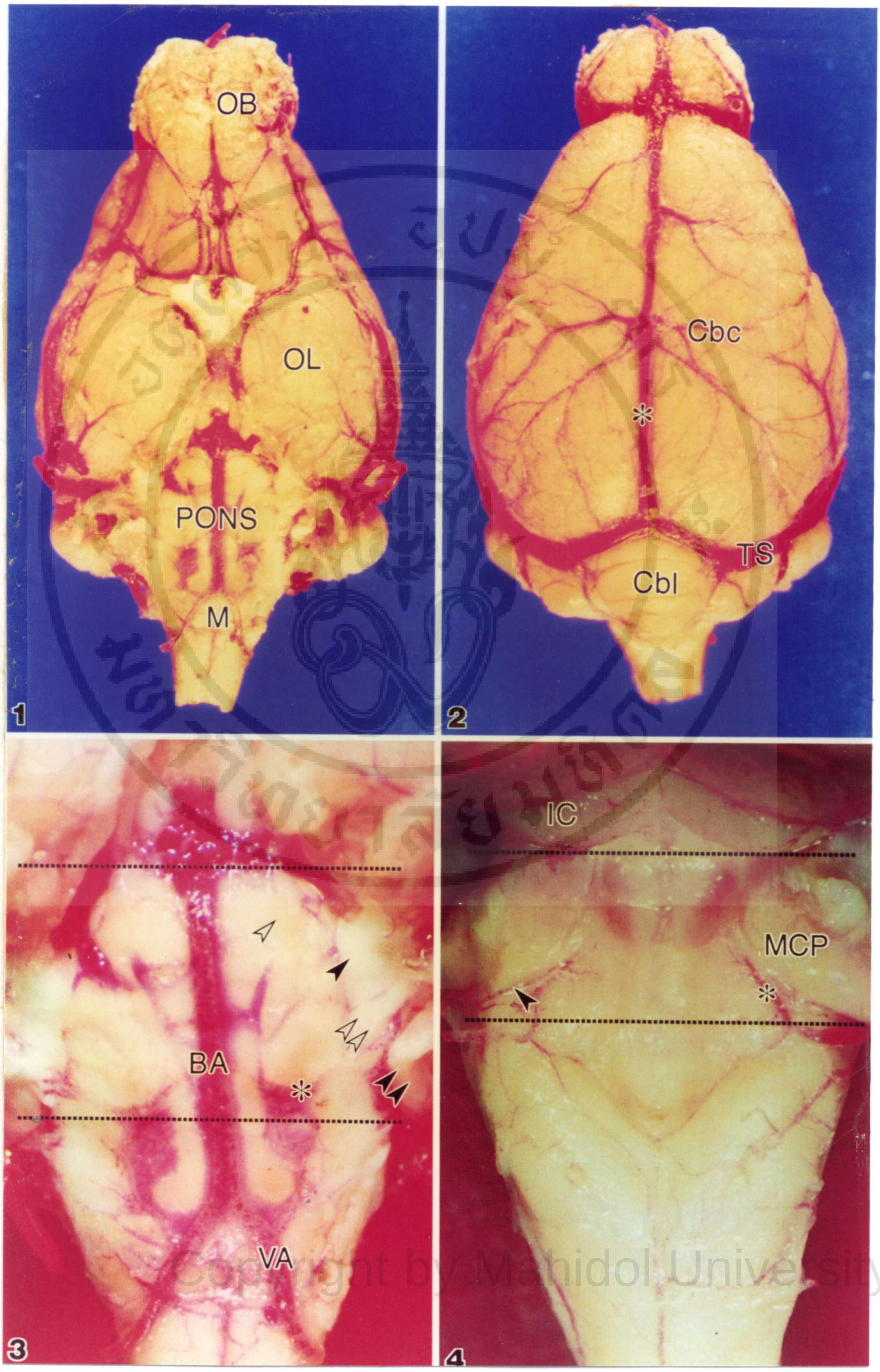
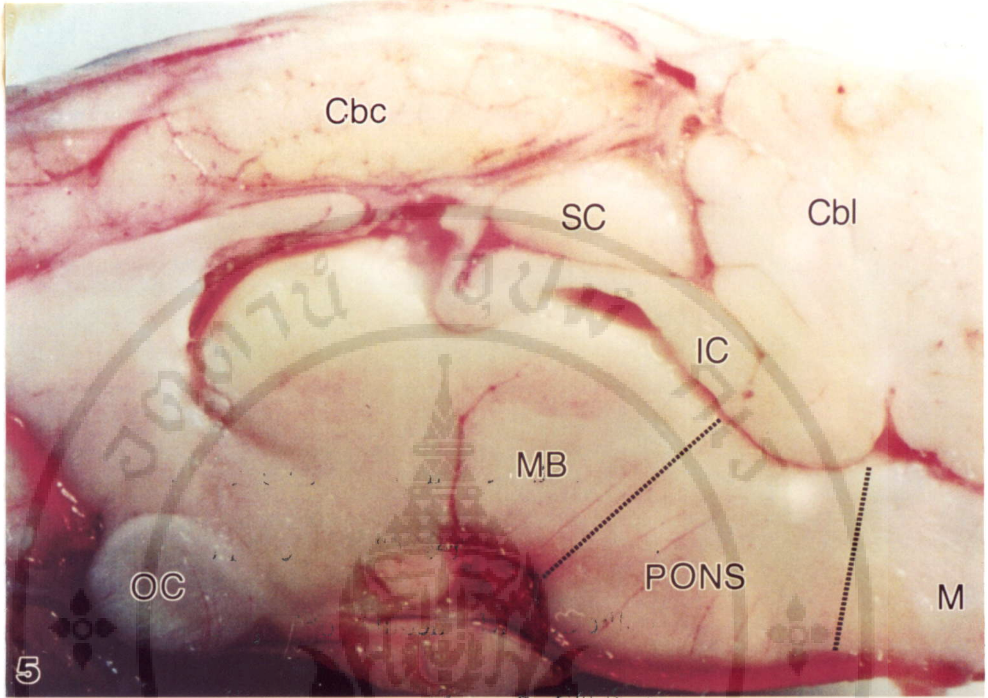

OB
OL
PONS
M
1
Cbc
*
TS
Cbl
2
BA
*
VA
3
IC
MCP
*
4

Figure 5. Stereomicrograph of the common tree shrew, sagittal section, showing the pons and related structures. Cbc, cerebral cortex; Cbl, cerebellum; IC, inferior colliculus; M, medulla oblongata; MB, midbrain; OC, optic chiasma; PONS, pons; SC, superior colliculus. 7.5X

Figure 6. Photograph of the arterial supply the pons of the common tree shrew coated with gold/palladium showing anterior inferior cerebellar artery (AICA), basilar artery (BA) and vertebral artery (VA); 1, rostral medulla artery; 2, inferior pontine artery; 3, middle pontine artery; 4, trigeminal artery; 5, rostral pontine artery; 6, superior cerebellar artery. 13X


Cbc
SC
Cbl
IC
MB
PONS
OC
M
5

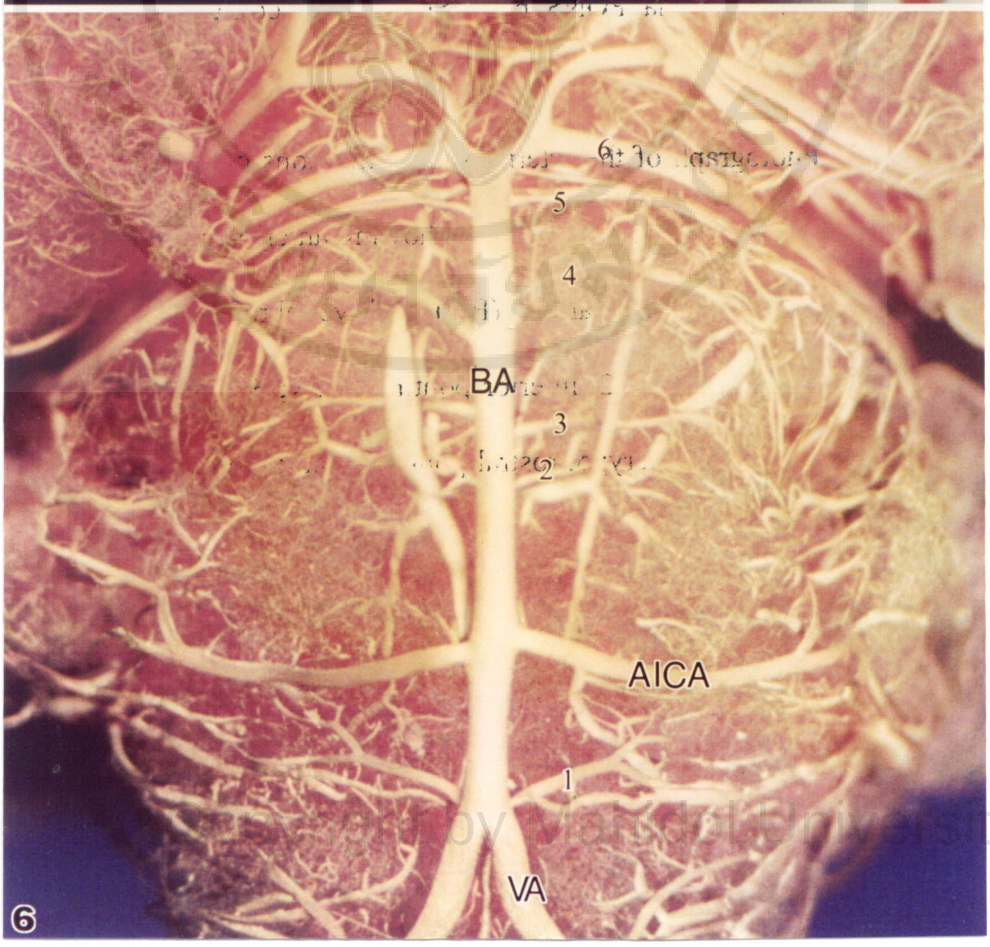
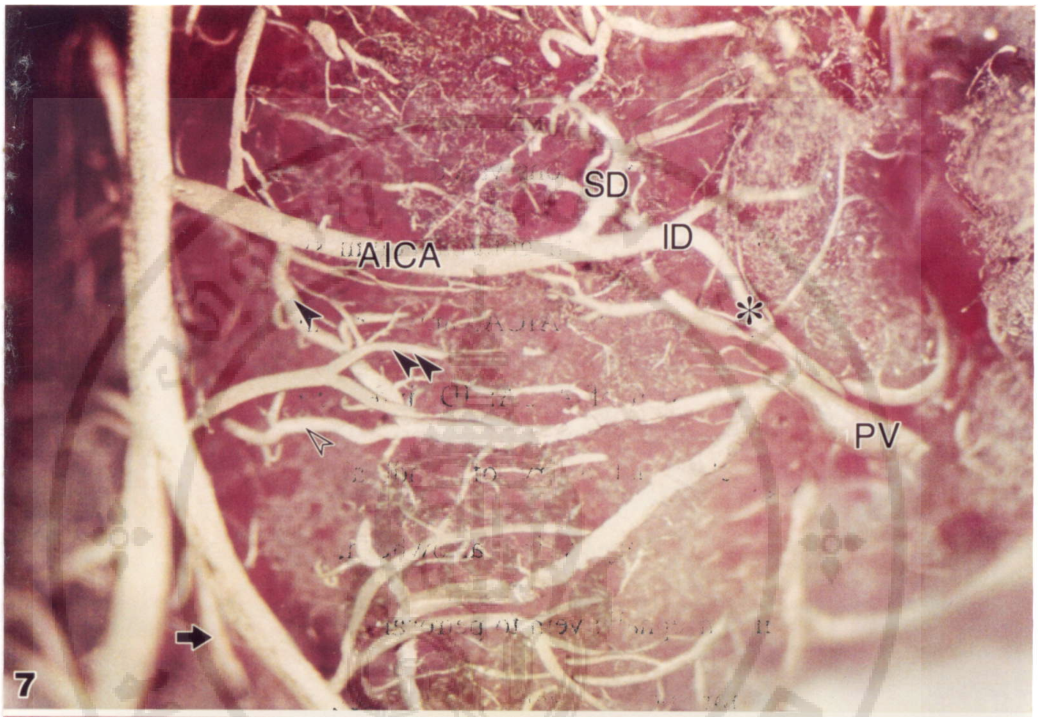

6
5
4
BA
3
2
AICA
1
VA
6

Figure 7. Stereomicrograph of pons vascular cast oblique view, in the common tree shrew coated with gold/palladium showing blood supply of the caudal part of pons. AICA, anterior inferior cerebellar artery; SD, superior division of AICA; ID, inferior division of AICA; asterisk, anterior choroidal artery of hindbrain; PV, petrosal vein; arrow, anterior medullary vein; arrowhead, connecting of anterolateral pontomesencephalic vein to petrosal vein; open arrowhead, connecting of anterior pontomesencephalic vein to petrosal vein; double arrowheads, caudal pontine artery. 15X

Figure 8. Stereomicrograph of the arterial of common tree shrew ventral view, coated with gold/palladium. BA, basilar artery; SCA, superior cerebellar artery; TA, trigeminal artery; arrowhead, inferior pontine artery; open arrowhead, middle pontine artery; asterisk, rostral pontine artery. 15X


SD
ID
AICA
*
PV
7

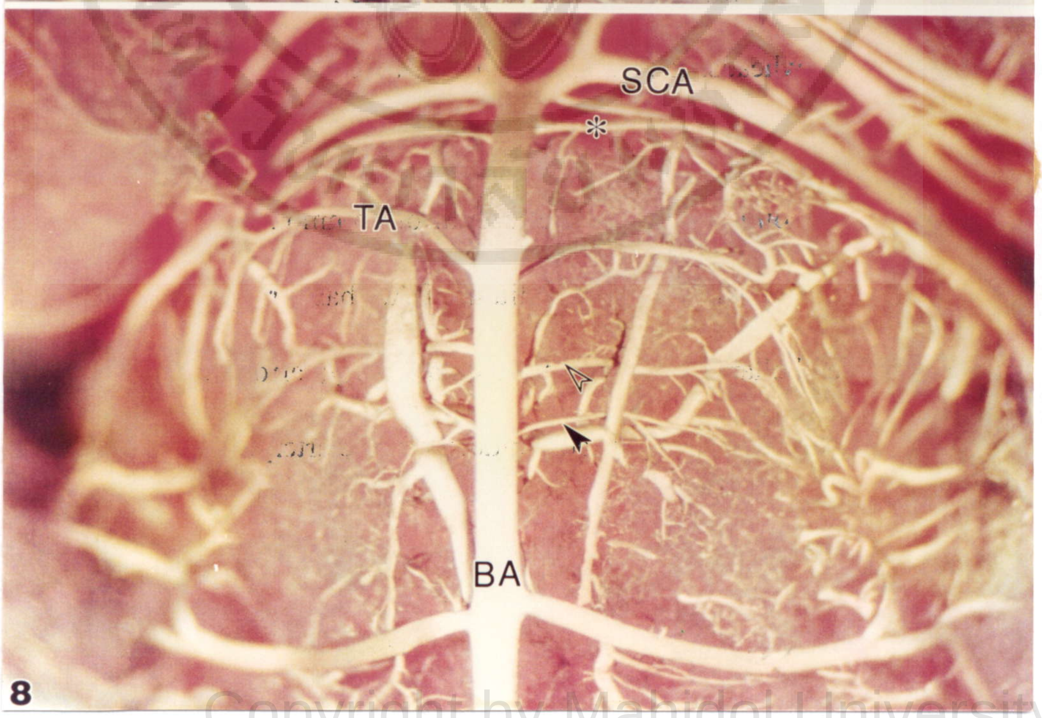
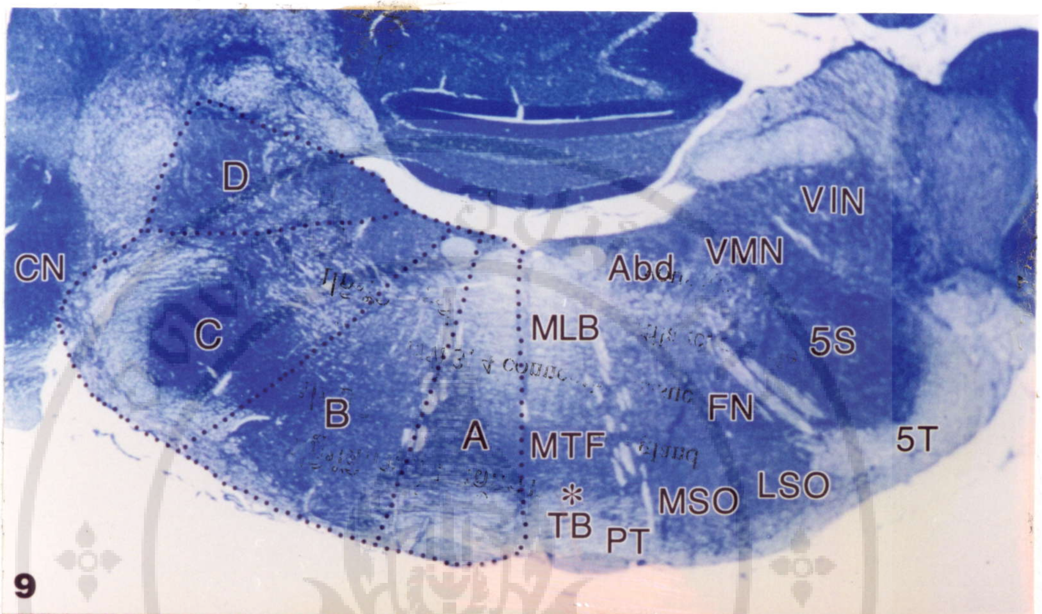
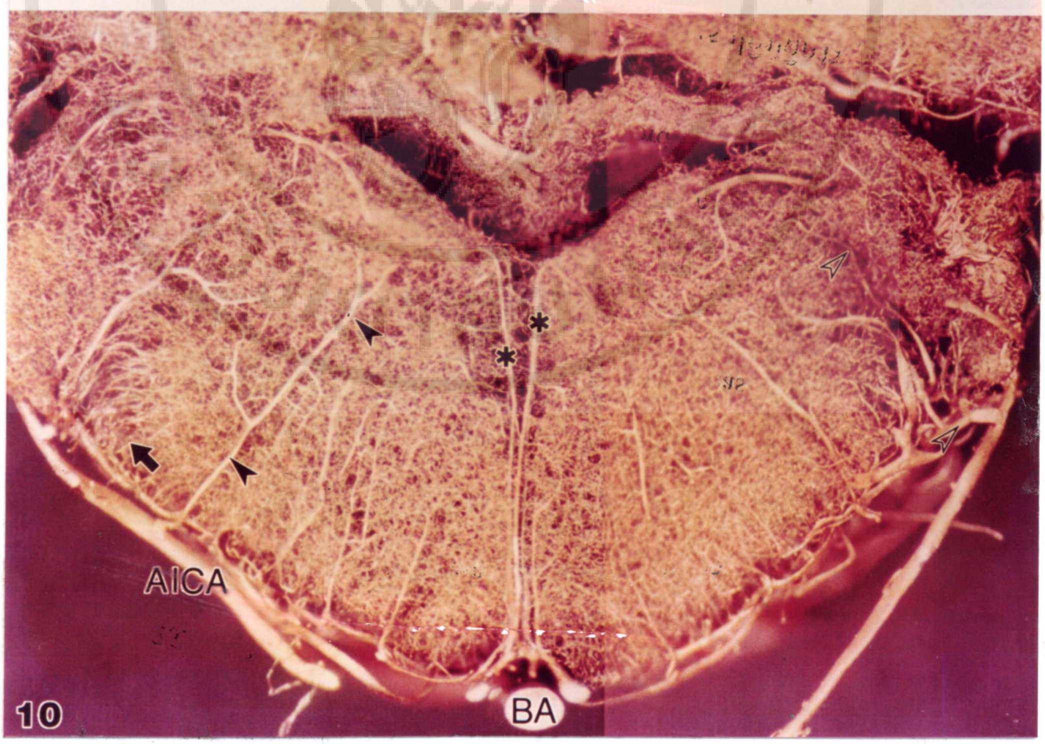

SCA
*
TA
BA
8

Figure 9. Light micrograph of the caudal pons, cross section, showing different areas of pons and nuclei. A, anteromedial area; Abd, abducent nucleus; B, anterolateral area; C, lateral area; CN, cochlear nucleus; D, posterior area; MT, medial tegmental field; FN, facial nucleus; TB, trapezoid body; asterisk, medial lemniscus; MLB, medial longitudinal bundle; PT, pyramidal tract; LSO, lateral superior olivary nucleus; MSO, medial superior olivary nucleus; 5S, spinal trigeminal nucleus; 5T, spinal trigeminal tract; VIN, inferior vestibular nucleus; VMN, medial vestibular nucleus. 20X

Figure 10. Stereomicrograph of the vascular cast of the caudal pons of the common tree shrew at similar level to that of figure 9 showing basilar artery (BA) and its branches. Asterisks, medial arteries; AICA, anterior inferior cerebellar artery; arrowhead, lateral branch of AICA; arrow, area of spinal trigeminal tract; open arrowhead, branch of AICA to vestibular and cochlear nuclei. 21X



9



10

Figure 11. Stereomicrograph of vascular cast of the caudal pons showing basilar artery (BA) and medial artery (asterisks). 24 X

Figure 11a Stereomicrograph of the vascular cast of caudal pons, ventral area, showing basilar artery (BA) and branch of caudal pontine arery (asterisk). 35X

Figure 11b Stereomicrograph of vascular cast of the caudal pons, dorsal area, showing branches deriving from medial artery (arrowheads). 45X

Figure 12 Stereomicrograph of vascular cast of pons, dorsal area, showing distal end of medial artery (open arrowhead), lateral branch of anterior inferior cerebellar artery (arrowhead) and branches of caudal pontine artery (double arrowhead). 35X

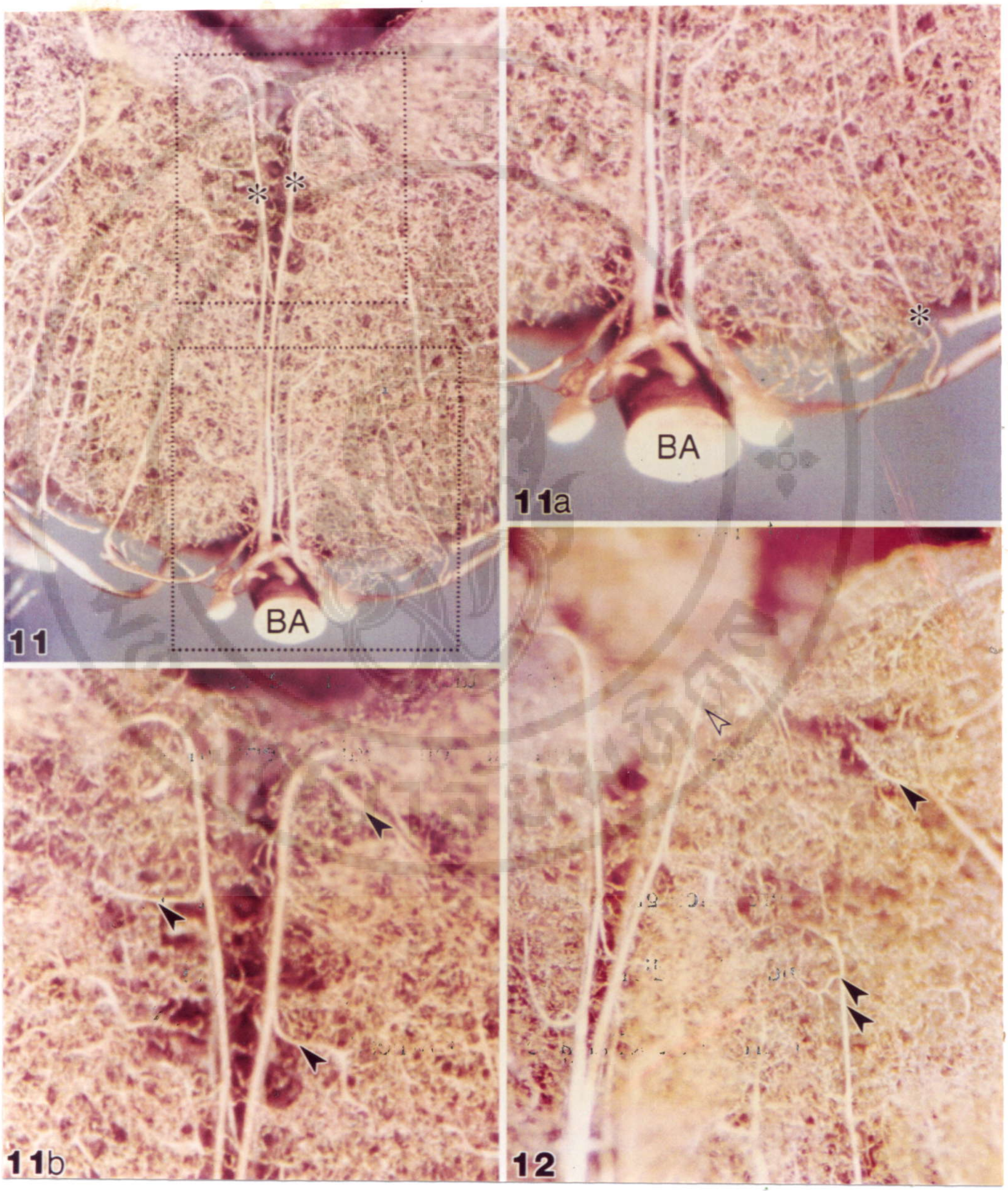
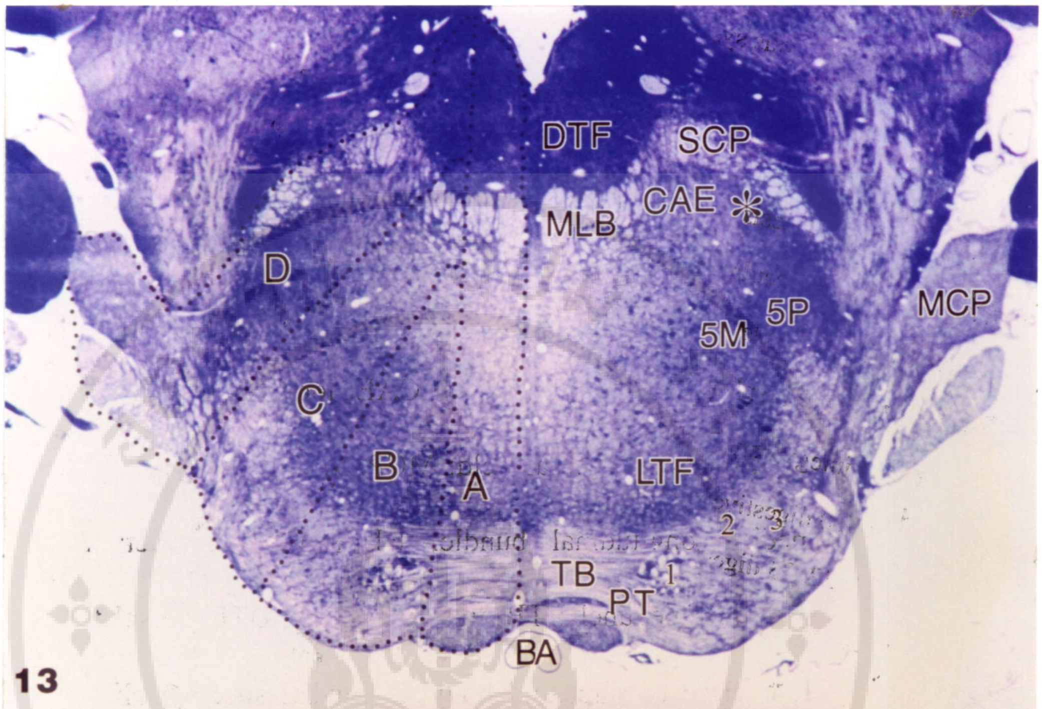

BA
11
BA
11a
11b
12

Figure 13. Light micrograph of rostral pons, cross section, showing various area of rostral pons and nuclei. A, anteromedial area; B, anterolateral; BA, basilar artery; C, lateral area; CAE, locus ceruleus; D, posterior area; LTF, lateral tegmental field; MCP, middle cerebellar peduncle; MLB, medial longitudinal bundle; PT, pyramidal tract; SCP, superior cerebellar peduncle; TB, trapezoid body; DTF, dorsal tegmental field; 1, nucleus of trapezoid body; 2, medial superior olivary nucleus; 3, lateral superior olivary nucleus; 5M, motor trigeminal nucleus; 5P, principal trigeminal nucleus; large asterisk, medial parabrachial nuclei. 20X

Figure 14. Stereomicrograph of the vascular cast of rostral pons showing superior cerebellar artery (SCA) and its divisions. BA, basilar artery; LD, lateral division of SCA; MD, medial division of SCA; RD, rostral division of SCA. 20X

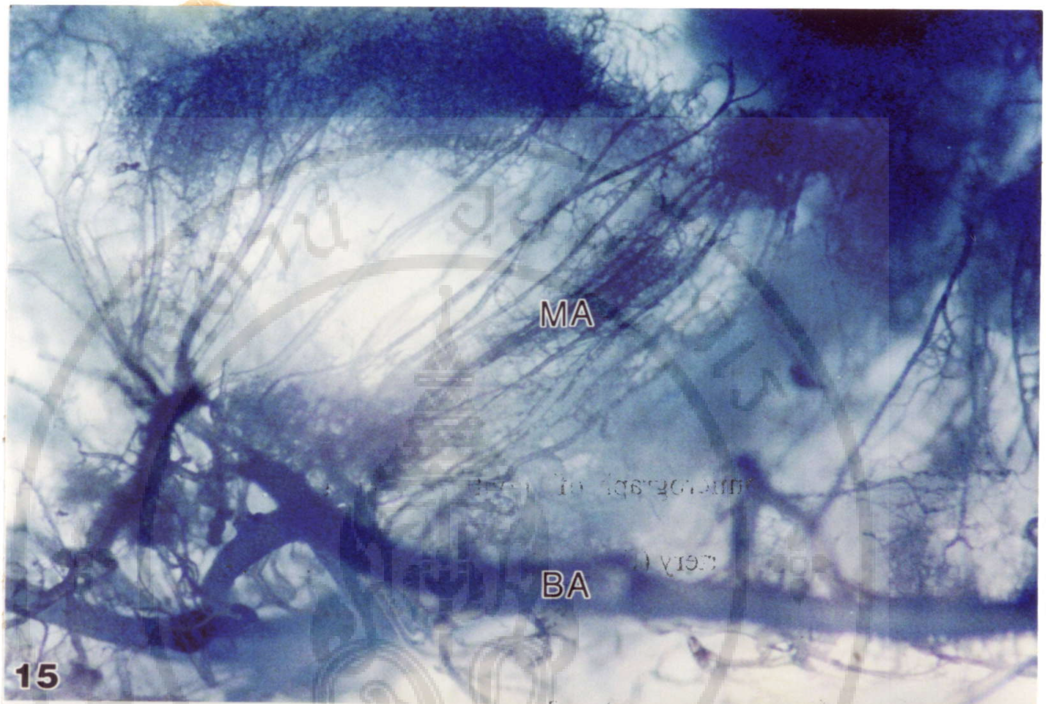


13

14

Figure 15. Stereomicrograph of pontine vascular cast, sagittal section, showing basilar artery (BA) and medial arteries (MA). 45X

Figure 16. Stereomicrograph of vascular cast of rostral pons showing basilar artery (BA), cerebral cortex (Cbc), medial artery (asterisk), superior cerebellar artery (SCA) and transverse sinus (TS). 10X


MA
BA
15

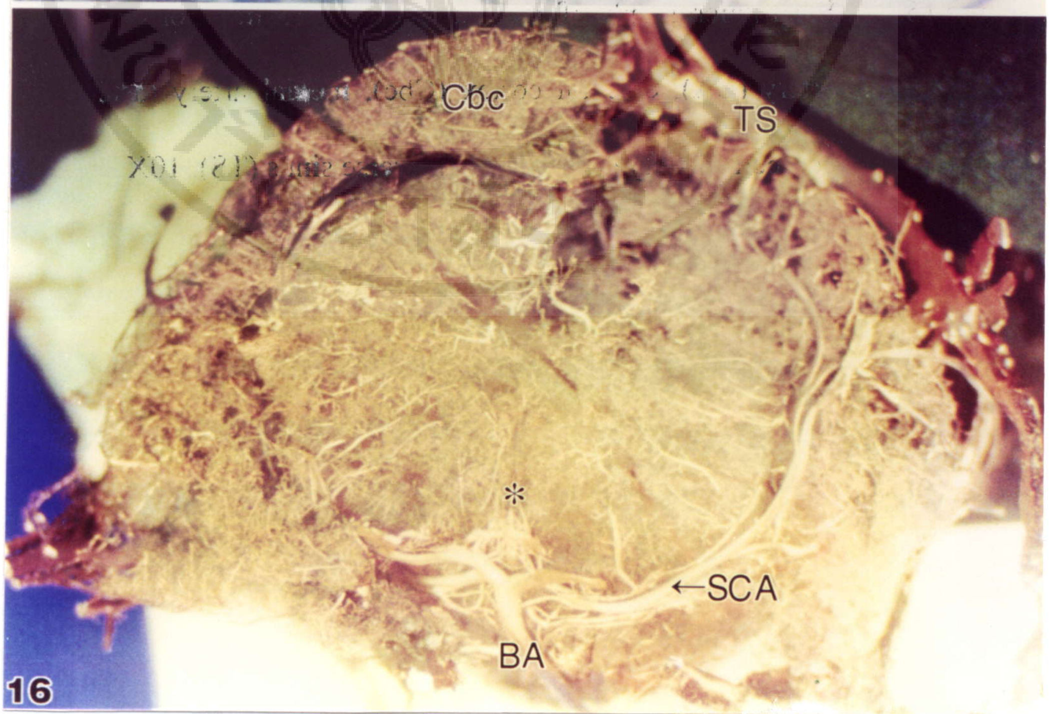

Cbc
TS
*
←SCA
BA
16

Figure 17. Stereomicrograph of vascular cast of rostral pons, caudal view, showing basilar artery (BA), medial arteries (open arrowheads) and paramedial arteries (arrowheads). SCA, superior cerebellar artery; asterisk, vein of pontomesencephalic sulcus. 30X

Figure 18. Stereomicrograph of rostral pons vascular cast, rostral view, showing right superior cerebellar artery (SCA) and branches of superior cerebellar artery which supply pons (arrowheads). MD, medial division of superior cerebellar artery. 40X

Figure 19. Stereomicrograph of rostral pons vascular cast showing left superior cerebellar artery (SCA) and its branches (arrowheads). 45X

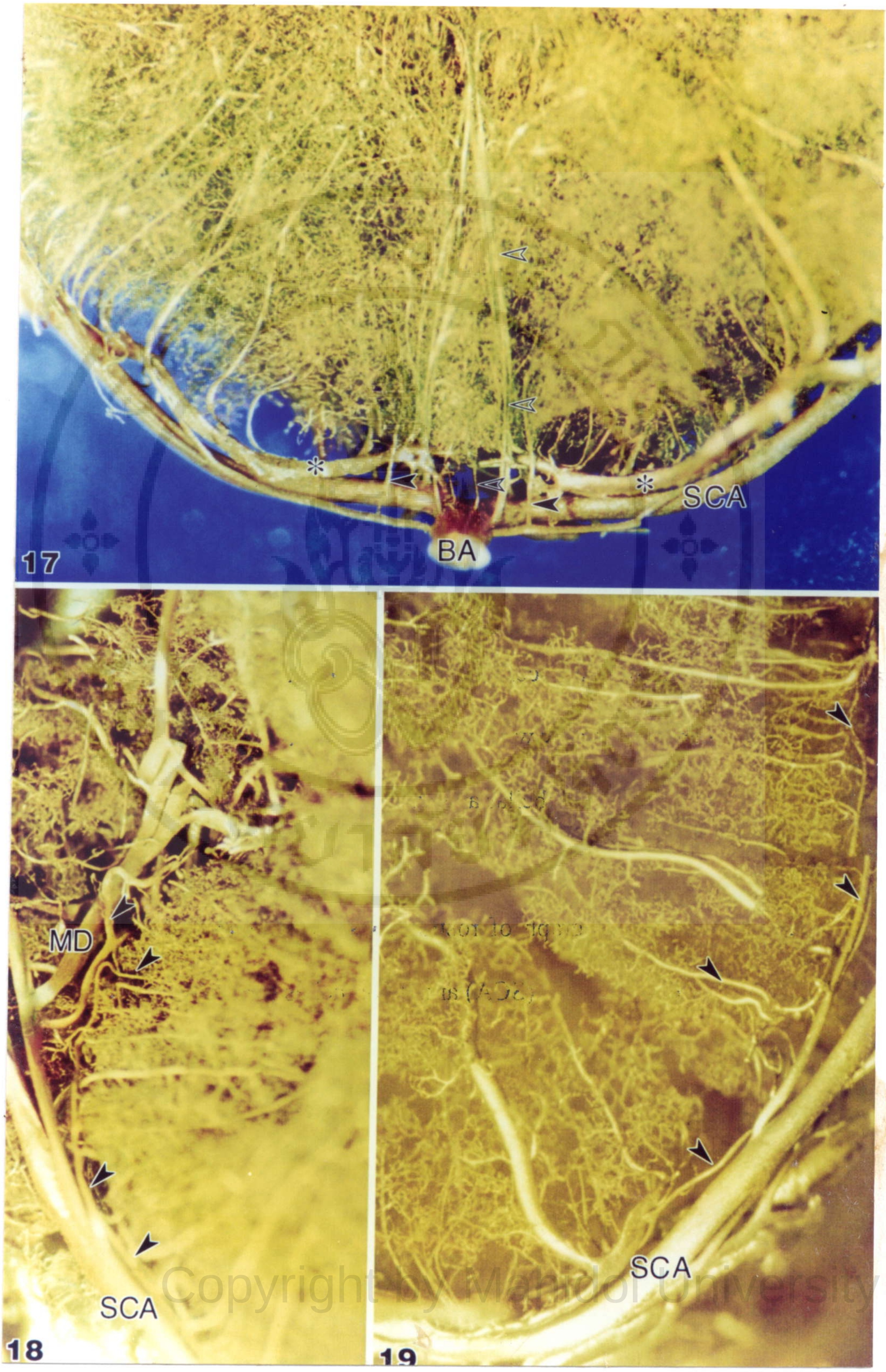

17
BA
SCA
18
MD
SCA
19
SCA

Figure 20. Stereomicrograph of pons vascular cast lateral view, showing medial arteries (arrowhead). AICA, anterior inferior cerebellar artery; BA, basilar artery. 45X

Figure 21. Stereomicrograph of pons vascular cast showing paramedial arteries (arrowhead) and vein of pontomesencephalic sulcus (asterisks). SCA, superior cerebellar artery; RPA, rostral pontine artery; arrowheads, paramedial arteries from rostral pontine artery; open arrowhead, anterolateral pontomesencephalic vein connected to vein of pontomesencephalic sulcus. 45X

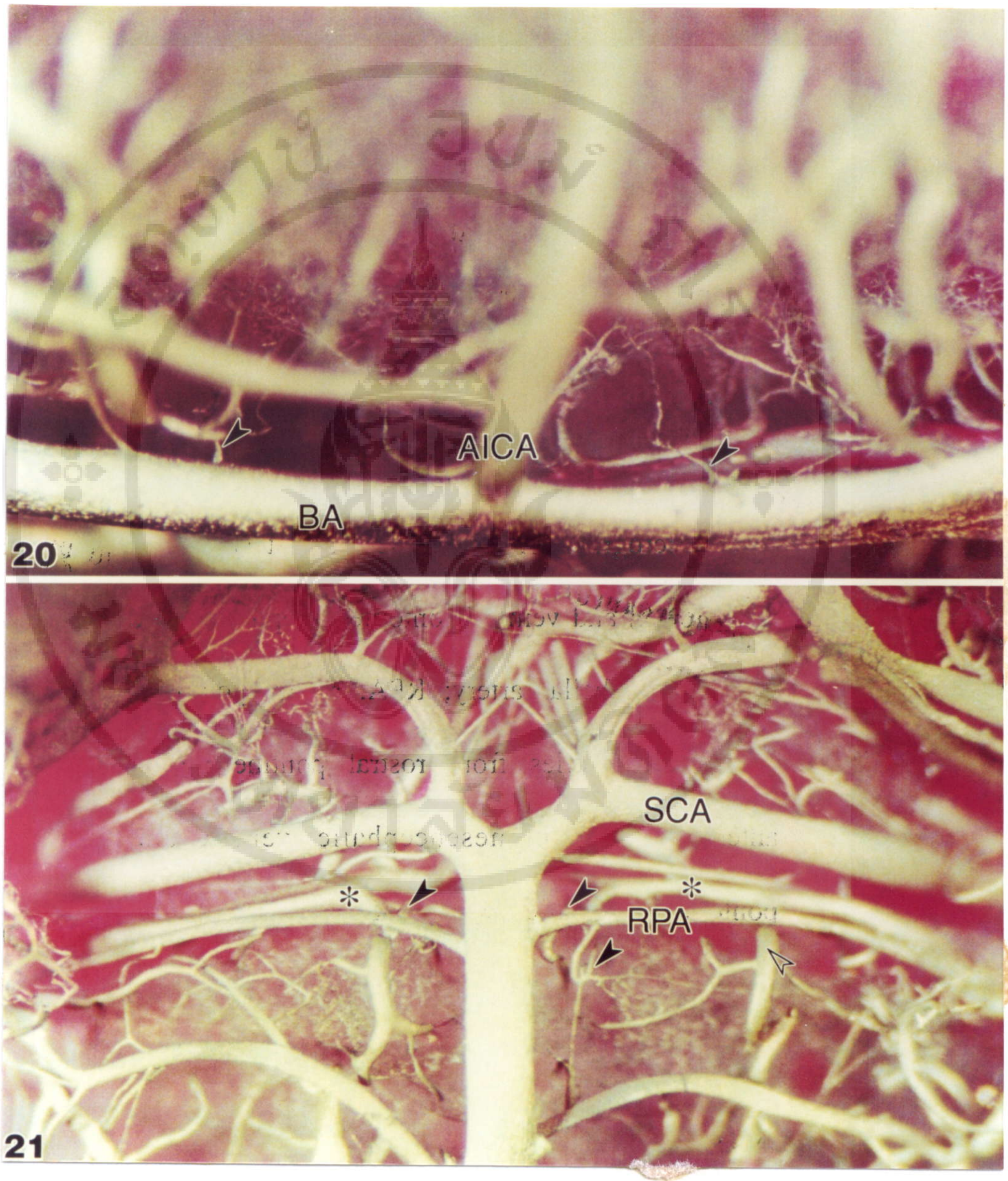

AICA
BA
20
SCA
RPA
21

Figure 22. SEM micrograph of vascular cast of the common tree shrew pons ventral view, showing basilar artery (BA) and its branches. MPA, middle pontine artery; RPA, rostral pontine artery; SCA, superior cerebellar artery; TA, trigeminal artery.

Figure 23. SEM micrograph of vascular cast of the common tree shrew pons ventral view, showing rostral pontine artery (RPA) and its branches. BA, basilar artery; arrowhead, arterial sphincter; open arrowhead, short branches of rostral pontine artery; double open arrowheads, long branch of rostral pontine artery.

Figure 24. SEM micrograph of vascular cast of the common tree shrew pons ventral view, showing anterior inferior cerebellar artery (AICA) and its branches. BA, basilar artery; Arrowhead, short branch of anterior inferior cerebellar artery; open arrowhead, long branch of anterior inferior cerebellar artery.

Figure 25. SEM micrograph of vascular cast of the common tree shrew pons oblique view, showing the right paramedial artery (arrowhead) derives from anterior inferior cerebellar artery (AICA). BA, basilar artery.

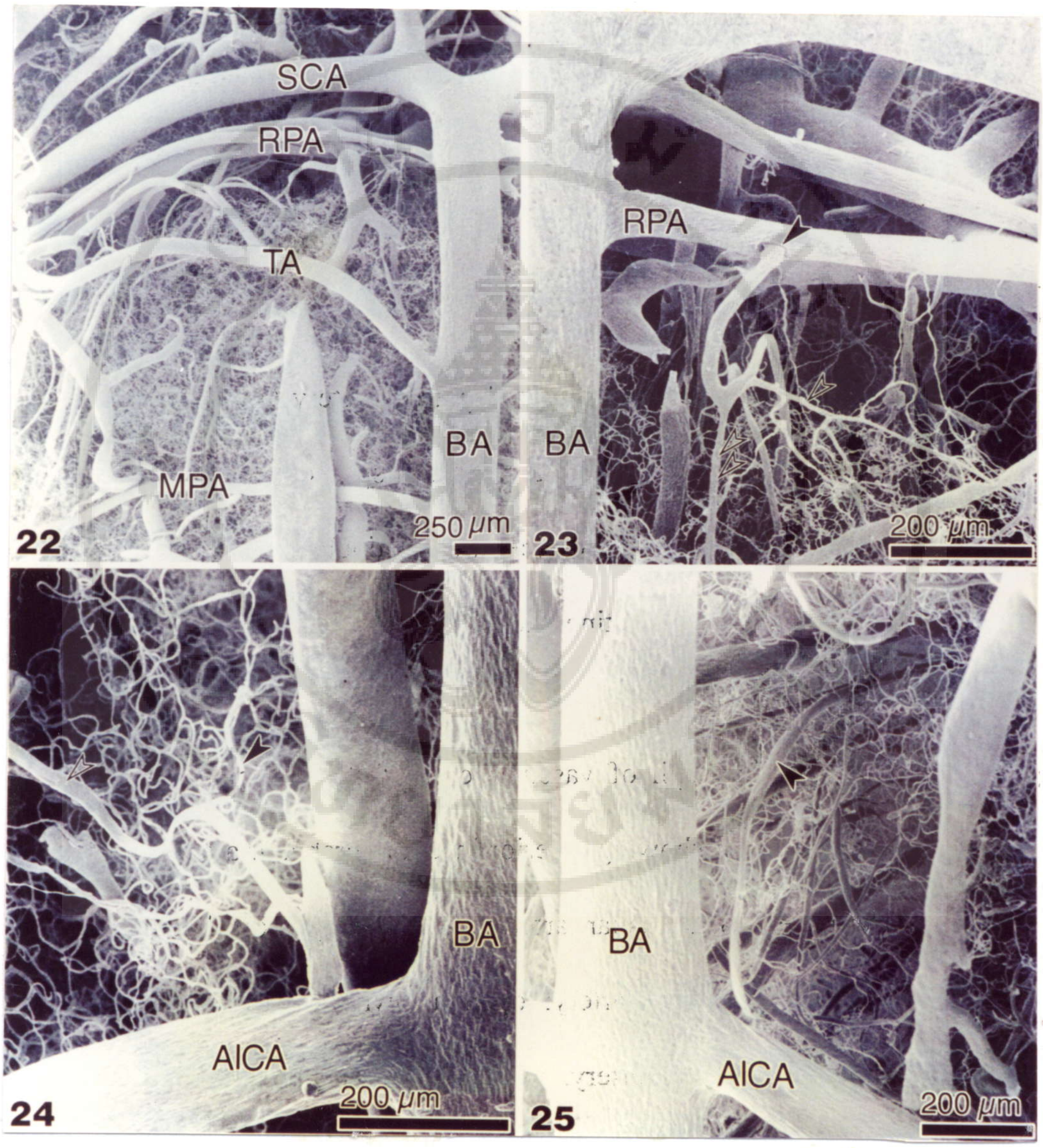

SCA
RPA
TA
BA
MPA
250 μm
22
RPA
BA
200 μm
23
BA
AICA
200 μm
24
BA
AICA
200 μm
25

Figure 26. SEM micrograph of vascular cast of the common tree shrew pons showing medial arteries (MA) and its branches. Asterisks, inverted branches of MA; arrow, branch of right MA to contralateral side; arrowhead, small medial artery; open arrowhead, tributaries of anterolateral pontomesencephalic vein; BA, basilar artery.

Figure 27. SEM micrograph of vascular cast of the common tree shrew pons showing tortuous branches of anterior inferior cerebellar artery. Arrowhead, short branch of AICA; open arrowhead, nuclear imprint.

Figure 28. SEM micrograph of vascular cast of the common tree shrew pons showing tortuous branches of AICA. Arrowhead, nuclear imprint; asterisk, smooth muscle cells wrapping around the artery.


MA
BA
26
50 μm
27
50 μm
28
50 μm

Figure 29. SEM micrograph of vascular cast of the common tree shrew pons ventral view, showing medial artery (MA) and the inverted branch (arrowhead).

Figure 30. SEM micrograph of vascular cast of the common tree shrew pons illustrating the non-fenestrated capillaries in pons (arrowheads).

Figure 31. SEM micrograph of vascular cast of the common tree shrew pons showing the dense capillaries network (double asterisks) in the area of trigeminal nuclei comparing with the area of trigeminal nerve tract (asterisk). Arrowhead, branches of AICA which supply trigeminal nucleus.

Figure 32 SEM micrograph of vascular cast of the common tree shrew pons showing the tributaries of vein which collect venous blood from capillaries network (arrowheads).

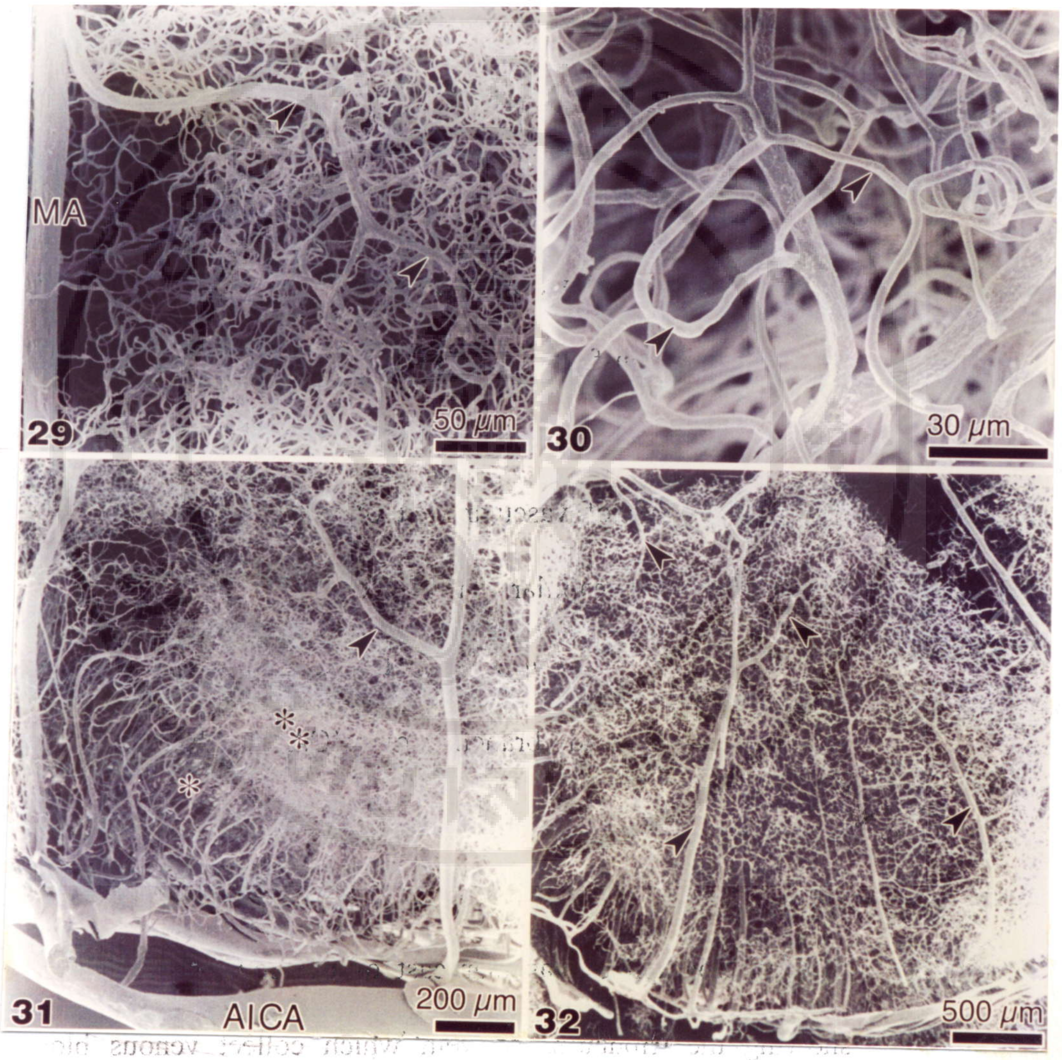
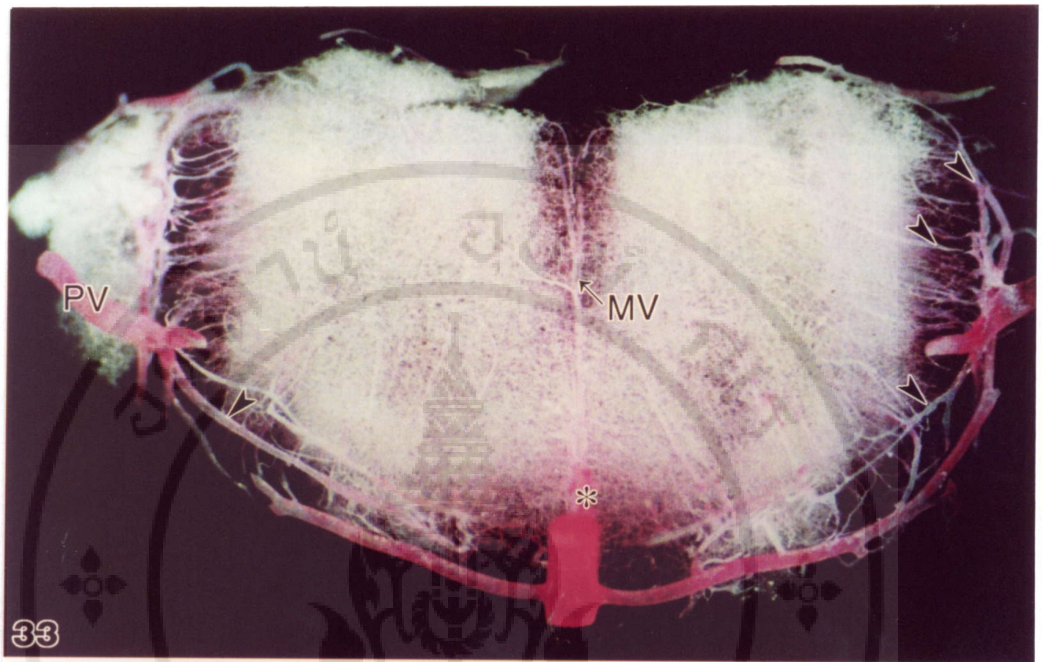

MA
29
50 μm
30
30 μm
31
AICA
200 μm
32
500 μm

Figure 33. Stereomicrograph of the vascular cast of caudal pons showing medial vein (MV) and anterior pontomesencephalic vein (asterisk). PV, petrosal vein; arrowheads, tributaries vein of petrosal vein. 20X

Figure 34. Stereomicrograph of the vascular cast of pons, sagittal section, showing precentral cerebellar vein (PcV), medial vein (MV), anterior pontomesencephalic vein (arrowhead) and basilar artery (BA). 7.5X


PV
MV
*
33

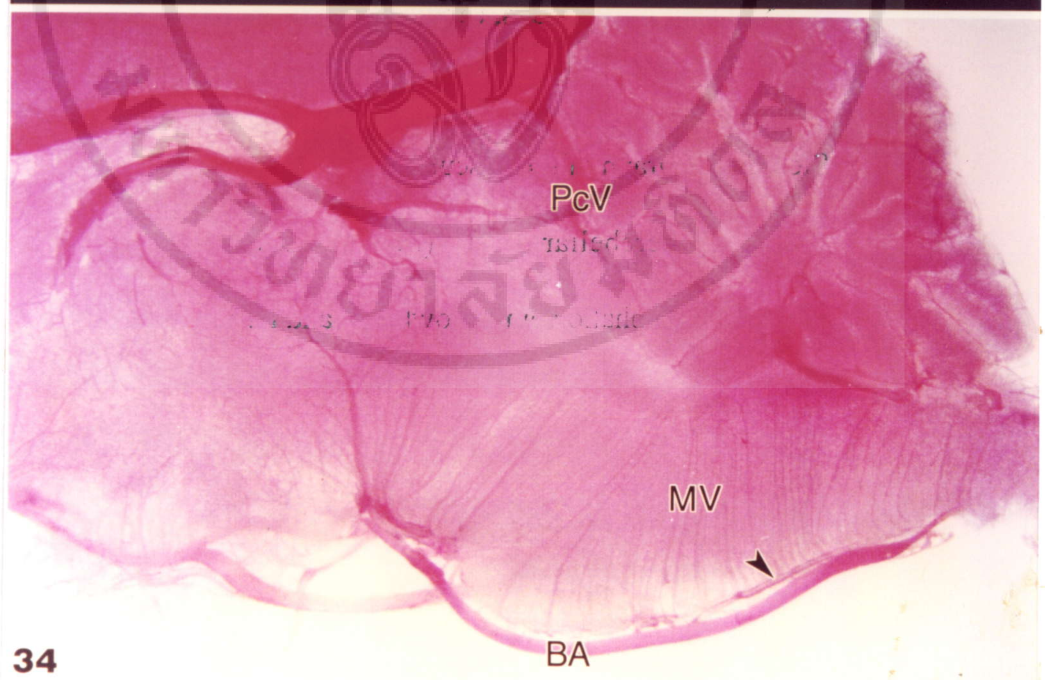

PcV
MV
BA
34

Figure 35. Stereomicrograph of vascular cast of pons, oblique view, showing anterior pontomesencephalic vein (arrowheads) and basilar vein (BV). 13X

Figure 36. Stereomicrograph of vascular cast of pons ventral view, showing venous drainage of pons. TP, transverse pontine vein; ALPMV, anterolateral pontomesencephalic vein; asterisk, vein of pontomesencephalic sulcus; arrowhead, connecting of anterolateral pontomesencephalic vein to vein of pontomesencephalic sulcus. 15X

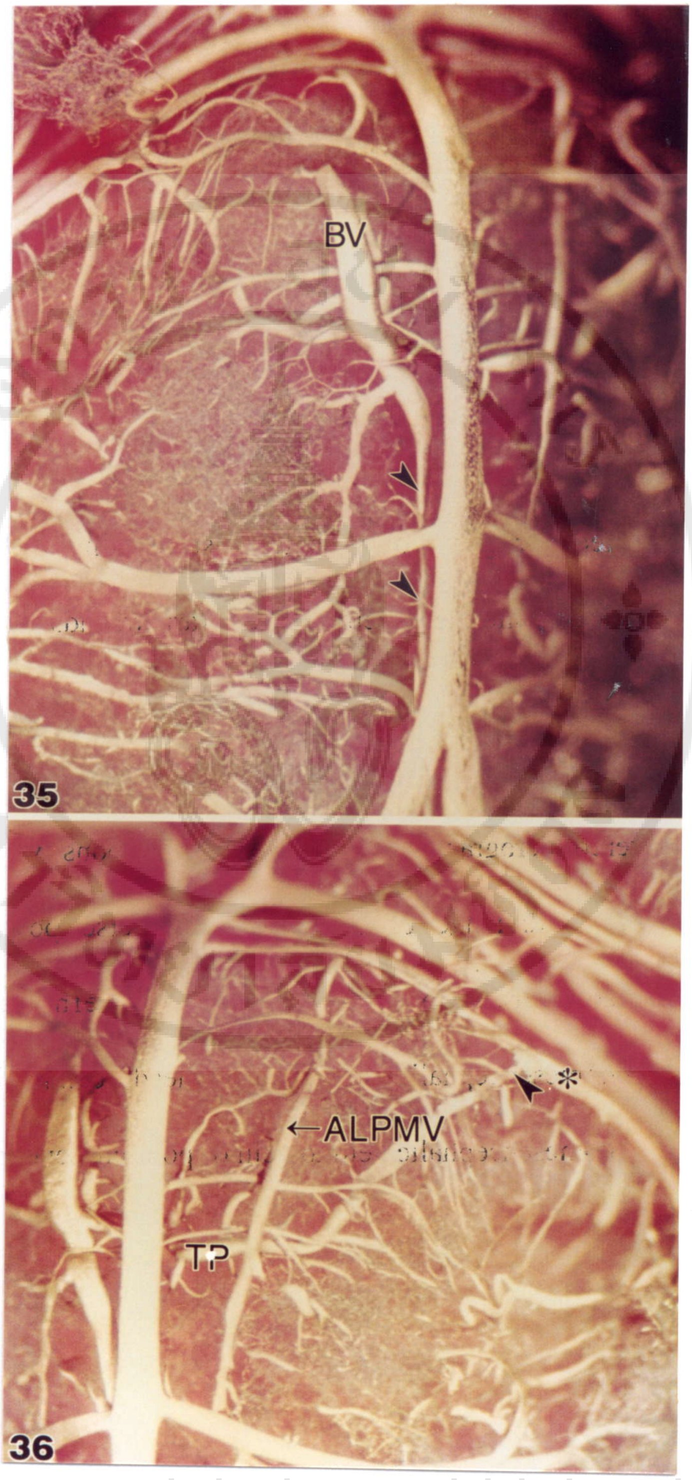
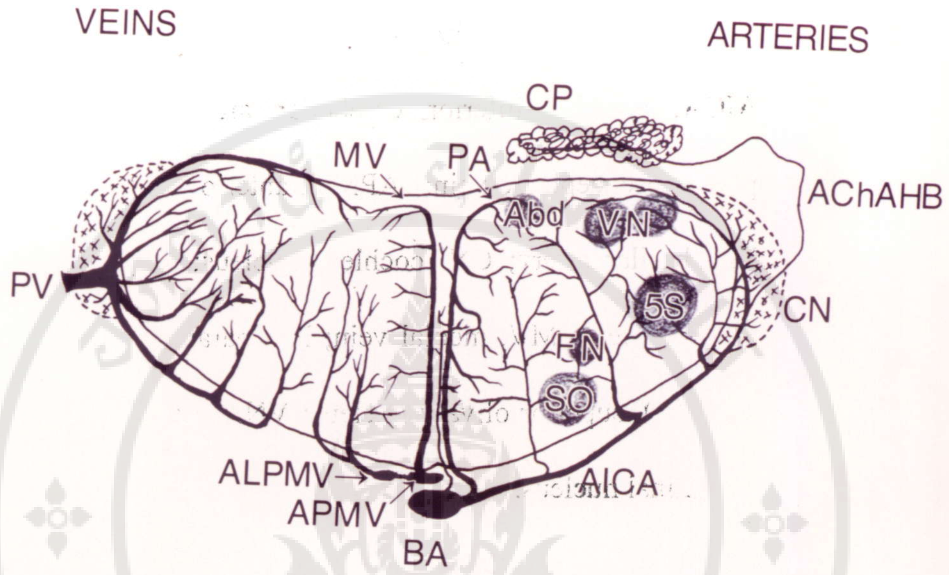
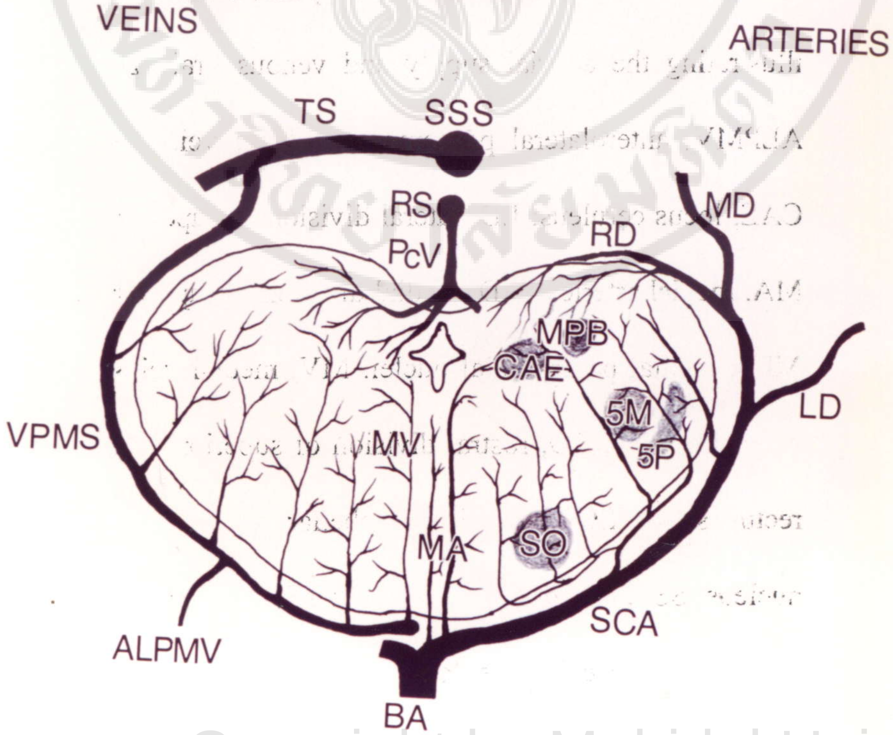

BV
35
←ALPMV
TP
36

Figure 37. Diagram of the common tree shrew pons, cross section of caudal part, illustrating the arterial supply and venous drainage of pons. Abd, abducent nucleus; AChAHB, anterior choroidal artery of hindbrain; AICA, anterior inferior cerebellar artery; ALPMV, anterolateral pontomesencephalic vein; APMV, anterior pontomesencephalic vein; BA, basilar artery; CN, cochlear nucleus; CP, choroid plexus; FN, facial nucleus; MV, medial vein; PA, paramedial artery; PV, petrosal vein; SO, superior olivary nucleus; VN, vestibular nucleus; 5S, spinal trigeminal nucleus.

Figure 38. Diagram of the common tree shrew pons, cross section of rostral part illustrating the arterial supply and venous drainage of rostral pons. ALPMV, anterolateral pontomesencephalic vein; BA, basilar artery; CAE, locus ceruleus; LD, lateral division of superior cerebellar artery; MA, medial arteries; MD, medial division of superior cerebellar artery; MPB, medial parabrachial nuclei. MV, medial veins; PcV, precentral cerebellar vein; RD, rostral division of superior cerebellar artery; RS, rectus sinus; SCA superior cerebellar artery; SO, superior olivary nucleus; SSS, superior sagittal sinus; TS, transverse sinus; VPMS, vein of pontomesencephalic sulcus; 5P, principal trigeminal nucleus; 5M, motor trigeminal nucleus.


VEINS
ARTERIES
CP
MV
PA
AChAHB
Abd
VN
PV
5S
CN
FN
SO
ALPMV
AICA
APMV
BA

37


VEINS
ARTERIES
TS
SSS
RS
MD
RD
PcV
MPB
CAE
5M
5P
LD
VPMS
MV
MA
SO
SCA
ALPMV
BA

38

CHAPTER V

DISCUSSION

As in the rat and armadillo, pons in the tree shrew is relatively small when compare to the whole brain (53, 54). The pons could be identified in the primitive and higher mammalian brainstem but not in the submammalian vertebrates as in reptile, fish, frog, and bird (55). The human pons is very large when compares to those of other mammals (53, 54, 56). The lower border of human pons is at the level of emerging point from the brainstem of the abducent nerve. It is the same level of the beginning of the basilar artery. In the rat, armadillo, opossum, dog and cat, the lower border of pons is also marked by the stem of abducent nerve. It is the same level of the beginning of the anterior inferior cerebellar artery (AICA) which is rostral to the beginning of the basilar (53, 54, 56). The lower border of the tree shrew pons is also at the level of AICA and corresponds to the caudal limit of the superior olivary nucleus when observed by light microscopy. The superior cerebellar artery is the landmark for the upper border of pons in the common tree shrew as in man (6, 70), rat (53), opossum (54), armadillo (54), dog (56) and cat (56). The striae medullaris pontis on the floor of the fourth ventricle is evident in man (6, 70) but could not be observed in the common tree shrew. At the ventrocaudal area, the pontomedullary sulcus is not demarcated in the common tree shrew eventhough it is present in man.

Furthermore, the trigeminal, abducent, facial, and vestibulocochlear nerves in the common tree shrew are originated from the pons as in man (6, 57), rat (53), opossum (54), armadillo (54), cat (56) and dog (56).

The Internal Structures of Pons

The pons of the common tree shrew could be divided into dorsal portion (the pontine tegmentum) and the ventral portion (the basis pontis) as in man (6), rat (58), cat (59) and dog (60). The basis pontis of the common tree shrew is very small and contains rather small pyramidal tract as in rat (58), cat (59) and dog (60) while in man (61) it is very large. This is reasonable since pyramidal fibers are responsible for motor function especially for that of the extremities in man. The position of the trigeminal, abducent, facial, vestibular, cochlear and superior olivary nuclei in the tree shrew are similar to that in man (61) and rat (53). In the lateral area, the size of the trigeminal nucleus and its tract in the common tree shrew, rat and dog is relatively small when compare to that of man (58, 60, 61). The abducent nucleus of tree shrew and of the rat is relatively large when compares to that of man (58, 61). This may indicate that, the common tree shrew and rat use their eyes intensively to search for food and locate the enemy. At the posterior area, the vestibular nucleus is large which suggests that the common tree shrew needs the vestibular nucleus to keep balance of the body when climbing or jumping among the trees. The common tree shrew has the relatively larger cochlear nucleus than man for it may need very effective hearing. This correspond to the large medial longitudinal bundle in the common tree shrew. However, size of the superior olivary nucleus is relatively large and similar to that of rat (58), cat (59) and man (61) but smaller than that of dog (60). The superior olivary nucleus could be divided into medial (MSO) and lateral superior olivary nuclei (LSO). It is well demonstrated in the cat that the MSO and LSO receive input from anteroventral cochlear nucleus (62). These input fibers are from the anterior (AA) and posterodorsal subdivision of the anterior division (PAD) of the anteroventral cochlear nucleus. In addition, the LSO also receives projections from the posterior subdivision (AP) of the anterior division and from the posterior division of the anteroventral cochlear nucleus (62). The projections to the LSO are bilateral whereas the projections to the LSO are almost ipsilateral (62). The LSO gives the projection fibers to the central nucleus of the inferior colliculus (63). In the central nucleus, a large medial component of projections end in par medialis and centralis. The small components end in the par lateralis. The MSO gives efferent projections mostly via the medial segment of the lateral lemniscus to the ventral division of the central nucleus of inferior colliculus (64). The superior olivary projections and input of the common tree shrew should be similar to those of the cat. The facial nucleus in the anteroventral area of the common tree shrew is located in the area similar to that of rat (58), cat (59) and man (61).

The Arterial Supply of Pons

In general, the organization of the major cerebral arteries of brain in the common tree shrew is similar to that in man. The main sources of the arterial supply of pons in the common tree shrew are from the basilar artery which is that in similar to rat (53), opossum (54), armadillo (54), dog (56), cat (56) and man (57, 70). The basilar artery is present in all of mammalian and submammalian vertebrates. However the size and shape of the artery are different (53, 54, 55, 56, 57, 58). The basilar artery in the dog, man, cat, rat, common tree shrew, armadillo and opossum has a uniform diameter throughout its length and the lateral rami arise asymmetrically and extend at variable distance over the pons. The basilar artery of the turtle (55) and dog (56) is tortuous but it is relatively straight in dogfish (55), frog (55), snake (55), chicken (55), cat (56) and man (70), The basilar artery in the tree shrew gives off branches namely, anterior inferior cerebellar, trigeminal, pontine, and superior cerebellar arteries as in man (6), rat (53) and cat (56). This artery in the dog, cat, rat and common tree shrew is relatively longer than in man. It situates on the ventral surface of both medulla oblongata and pons. Furthermore, the superior cerebellar artery is a branch of the basilar artery in man but it is from the posterior communicating in the dog (23). The anterior inferior cerebellar artery in the common tree shrew is the main branch to supply the caudal part of pons. The labyrinthine artery is the branch which deriving from the AICA in common tree shrew but it is the direct branch from basilar artery in man to supply the inner ear (38). The trigeminal artery is present in all submammals as well as mammals (54). This artery gives off branches to supply trigeminal nucleus, tract, and ganglion as in man. The superior cerebellar artery of the common tree shrew is the main arterial supply to the cerebellum. It also gives off branches to supply the rostral part of pons as in man (6, 65, 66), rat (53), armadillo (54), opossum (54), dog (56) and cat (56). The pontine branches of basilar artery supplies the arterial blood to anteromedial, anterolateral and lateral areas of pons as in guinea pig (20, 28), rat (53), armadillo (54), opossum (54), cat (56) and dog (56).

The intrinsic arteries of tree shrew came from the pontine surface penetrating into the brainstem are in the same zonal pattern as in man and dog (56). In the caudal part at the anteromedial area of the common tree shrew, the pyramidal tract, trapezoid body, medial longitudinal bundle, medial lemniscus and abducent nucleus are supplied by the medial, paramedial and pontine branches of the basilar artery. It is noted that the medial longitudinal bundle in common tree shrew, rat (58), cat (59), dog (60), and is relatively large than that in man (61). This indicates that there is a large number of fibers in the bundle. In the tree shrew, the superior olivary, facial, trigeminal nuclei and its tract and cochlear nuclei are supplied by branches of AICA. At the posterior area, the vestibular nucleus is also supplied by branches of AICA. The findings of this study are similar to those in the guinea pig (20, 28) and human brainstem (22). All areas in the rostral part of tree shrew pons are supplied by branches of SCA and pontine branches of basilar artery. The motor trigeminal nuclei, the locus ceruleus and the parabrachium nuclei are also supplied by the rostral division of SCA as in man (28, 65, 66). It is observed that the dense vascular net is noted in the areas represented by nuclei or groups of neurons. They are the superior olivary, abducent, trigeminal, vestibular and cochlear nuclei. The lower density of blood supply is observed in the areas containing fibers or nerve tracts such as trigeminal, pyramidal tracts, medial longitudinal bundle, superior and inferior cerebellar peduncles as has been documented in guinea pig (20, 28). It is observed in the tree shrew that, the proximal part of the secondary branches from the basilar artery are tortuous, curve, wavy or spiral and becoming rather straight before penetrating into the pons. These features are noted in almost all levels of pons. It is possible that the arterial branches need the extra elongation in order to subserve the physical strength at the interface between the vascular turn and the pons. The reserve length is likely to reduce the sheer stress. It may also need to modulate the arterial pressure by becoming tortuous to increase the resistance of the blood vessel. It is also found that, the proximal portion of the small arterial branches deriving from the large branch are with arterial sphincters to control the blood flow pressure and volume. It is observed that the medial arteries near the ventral midline of the pons are the branches deriving directly from the left and right posterolateral aspect of the basilar artery before penetrating into the paramedial line into pons. They do not come from the midline of the posterior aspect of basilar artery while the paramedial arteries are derived from pontine branches of the basilar artery and these branches are not direct branch of the basilar artery. This may be that the pia mater in the midline is thicker than that in the paramedial line. There are branches deriving from the medial and paramedial arteries in right and acute angle. Some branches are coursing in inverted direction from the floor of the fourth ventricle to the ventral area of pons. It is likely that the areas which are supplied by these branches are developed and rapidly extending in the ventral direction so the arteries that supply them are displaced. It is also obvious that the ventral part of pons is very thick. The inverted branches are from the distal ends of the medial and paramedial branches. They are curving and returning to the ventral area of pons before breaking into arterioles and capillary networks. These capillaries anastomose with those from other arterial branches to form the capillaries network to supply the nuclei in the anteromedial, lateral and posterior areas. In the common tree shrew, the penetrating arteries of pons exhibit the radial pattern as appear in midbrain (47) and similar to guinea pig (20, 28) and man (22). It is noted that the capillaries in the pons are with out fenestration as in the other part of the brain (47, 77, 78). This indicates that the blood vessels should be well established of blood brain barrier in the pons.

The Venous Drainage of Pons

The venous drainage of the pons in the common tree shrew is quite similar to that in man (25, 26, 27, 67, 68, 69) but somewhat different from guinea pig (28). In the guinea pig, most of venous blood are drained into the basilar vein (28). In man and the common tree shrew, the venous drainage in the pons could be divided into anterior or petrosal, superior or galenic and posterior group (27, 68). It is noted that the petrosal vein in the common tree shrew drains the venous blood into inferior petrosal sinus while into superior petrosal sinus in man (27, 70). The vein of pontomesencephalic sulcus in the common tree shrew is large. It collects the venous blood from anteromedial, anterolateral and lateral areas of rostral pons and drains directly into transverse sinus while the small amount of blood is drained into lateral pontomesencephalic vein and further into great vein of Galen as in man (67, 70). The precentral cerebellar vein or collicular vein in the tree shrew collects the venous blood from posterior area of rostral pons to drain it into rectus sinus. This is different from the case of man in that this vein flows into the great vein of Galen (27, 67).

So far, the tree shrew has been classified as a member of the Order Insectivores (49, 71) or of Order Primate (48, 50, 72, 73, 74, 75). In this study, the anatomy and histology of pons in the common tree shrew (*Tupaia glis*) can not be used as good criteria to classify this animal as Insectivores, Primate or in a separate order as Order Scandentia (76). However, the results of this study indicate that the pontine microvascularization in the common tree shrew (*Tupaia glis*) is very similar to that of man.

CHAPTER VI

CONCLUSIONS

The microvascularization of pons in the common tree shrew (*Tupaia glis*) has been studied by vascular corrosion cast technique with stereomicroscope and scanning electron microscope (SEM). The findings are:

I. The arterial supply of the pons

1. The main blood supply of pons is from basilar artery and its branches which are RMA, AICA, IPA, MPA, TA, RPA and SCA.

2. The arteries give off branches penetrating into the internal part of the pons in radial pattern of centripetal arrangement with the direction toward the fourth ventricle.

3. The areas of blood supply by these arteries could be divided into anteromedial, anterolateral, lateral, posterior areas according to the points of penetration and the corresponding territories that they supply.

4. The medial arteries originating from basilar artery are derived from left and right posterolateral aspect of basilar artery. None of these arteries is derived from the midline of the posterior aspect of basilar artery.

5. The branches of pontine arteries are tortuous before breaking into short and long branches to supply the pons.

6. The degree of vascularity in the pons is high in the area accupied by the pontine nuclei and low in the area containing nerve fibers or tract.

7. The capillaries in the pons are without the fenestration.

II. The venous drainage of the pons

1. The venous drainage could be divided into the anterior or petrosal group and the posterior group.

2. The venous blood from anteromedial area is collected into the anterior pontomesencephalic vein and further into transverse pontine vein. The venous blood from anterolateral area is drained into anterolateral pontomesencephalic vein and further into transverse pontine vein before proceeding into vein of pontomesencephalic sulcus rostrally and into petrosal vein caudally.

3. The venous blood from lateral and posterior areas is collected into tributaries of petrosal vein which drains into petrosal vein then further into inferior petrosal sinus.

4. The venous blood from anteromedial and anterolateral areas of the rostral pons is drained into the vein of pontomesencephalic sulcus, ventrally. The venous blood from the posterior area of rostral pons is collected into precentral cerebellar vein dorsally and further into the rectus sinus.

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APPENDIX

APPENDIX I

The Common Tree Shrew (*Tupaia glis*)

Taxonomy

Kingdom	:	Animal
Phylum	:	Chrodata
Subphylum	:	Vertebrata
Class	:	Mammalia
Subclass	:	Eutheria
Order	:	Primate
Suborder	:	Prosimii
Family	:	Tupaiidae
Subfamily	:	Tupaiinae
Genus	:	Tupaia
Species	:	glis

Synonyms

Sorex glis Diard 1820

Tupaia ferruginea Raffes 1821

Cladobates belangeri Wegner 1841

Tupaia peguanus Lesson 1842

Sciurus dissimilis Ellis 1860

Tupaia chineusis Anderson 1879

Tupaia picta Thomas 1892

Tupaia phaeura Miller 1902

Tupaia chrysogaster Miller 1903

Tupaia carimatae Miller 1906

Tupaia modesta Allen 1906

Tupaia discolor Lyon 1906

Tupaia concolor Bonhote 1907

Tupaia lacernata Thomus and Wroughton 1909

Tupaia obscura Kloss 1911

Tupaia natunae Lyon 1911

Tupaia belanaeri Thomas 1914

Tupaia conedor Kloss 1916

Tupaia clarissa Thomas 1917

Vernacular Names

Spitzhornchen

Common tree shrew

Tupaia ferrugineux

Painted tree shrew

Mill's tree shrew

Diagnosis

"Tupaia " is derived from the Malay " tupaia ", meaning squirrel- like animal. Tree shrew can be distinguished from squirrels in the field by their long, pointed muzzle and behavior less arboreal than their common name would suggest. The common tree shrew is semi-arboreal and feed extensively on the ground. The characteristics of the tree shrew are:

Size (mm)		Male	Female
head to body	:	160-230	160-240
tail length	:	140-195	140-190
Weight	:	85-185 gm	
Fur	:	greybrown, finely speckled with black	
Cranium	:	primate-like, rounder, elongated muzzle and small Brain case, laterally directed orbits but show a post-orbital bar	
Eye	:	relatively big, completely encircled by bone	
Ear	:	thick, small and quite human in form	
Nose	:	elongated shrew-like nose terminating in a necked moist snout	
Body	:	squirrel-like animals	

Hand & Feet	:	5 fully-formed digits on each hand and foot, all digits bear claws, no nails and not fully apposible well marked pads on palm
Tail	:	long and slender, well long hair on dorsal surface with undersurface lacking long hairs
Mammae	:	two, four or six mammae
Chromosome	:	2n = 60-62

Distribution

From Nepal and Sikkim, East to South China and throughout Southeast Asia to Indonesia but not in the Philippines. There are 8 mainland Thai subspecies : Tupaia glis ferrugonea, Tupaia glis wilkensoni, Tupaia glis clarissa, Tupaia glis belangeri, Tupaia glis chinensis, Tupaia glis laotum, Tupaia glis olivacea and Tupaia glis concolor.

Ecology and Behavior

The common tree shrews actually spend much of their time on ground, foraging on the forest floor and generally omnivorous, eating anything they come across, including ants, termites, beetles, fruit, spiders, seeds, bugs and even lizards and small rodents. There is no evidence that they shovel through the forest litter as shrews do.

Tree shrew are nervous, aggressive animals. Males will not tolerate the presence of other males, though there seems to be little fighting between the sexes. They typically from pairs which are strongly territorial, and follow the same pathways within their territory. They are found of water and often bathe in water-filled hollows of trees.

There is no fixed birth season in captivity, pregnancies have been observed to be associated with the period of rainfall; June, July and August. Nests are built in holes in fallen trees, hollow bamboos or similar sites. The gestation period is approximately 41-50 days. The numbers of young are 1-4 (usually 2). Newborn is pink, hairless and has dosed eyes. Pigmentation appears on the fourth day; hair begins to grow on the fifth day. Teeth begin to appear about the eleventh day and the eyes open on the twenty fifth day. At 6 months, they are sexually mature. Longevity is 2-3 years, with maximum of 5.5 years in captivity. (Napier; 1967, Martin; 1966, Lekagul; 1977).

The main similarities between tree shrews and primates

Skull	: snout relatively short, postorbital bar present, pattern of bones in medial orbital wall, enlarged braincase, advanced form of auditory ossicles
Dentition	: tooth-come present in front of lower jaw
Limbs	: highly mobile, ridged skin on palms and sloes

Brain and sense organs	:	olfactory apparatus reduced, visual apparatus enhanced, central avascular area of retina, neocortex expanded, calcarine sulcus present
Reproductive	:	pendulous penis, scrotal testes, discoidal placenta, small number of teats
Miscellaneous	:	caecum present

APPENDIX II

Batson's # 17 Plastic Mixture for Vascular Casting

Plastic Mixture Preparation

Batson's # 17 Monomer base solution*	12.5 ml
Batson's # 17 Catalyst*	3.5 ml
Batson's # 17 Promoter*	0.5 ml
Acron Denture Base **	6.5 ml

Prepared in an ice bath, the solution are mixed thoroughly and used immediately.

* Batson's corrosion kit can be obtained from:

Polyscience, Inc.

Paul Valley Industrial Park

Warring, Pa. 18976 USA

** Acron Denture Base can be obtained from:

Yoawarach

Bangkok, Thailand

APPENDIX III

Bouin's Solution

For histological study, the bouin's solution was used to preserve the brain tissue.

This fixative contains :

Picric acid (saturated aqueous)	75 ml
Formaldehyde 40%	25 ml
Glacial acetic acid	5 ml

Picric acid is a bright yellow crystalline substance, usually supplied damped with water because of its explosive property if heated. It is slightly water soluble (about 1% at room temperature) but more soluble in alcohol (about 5%) and benzine (10%). Picric acid precipitates nucleoprotines and causes little shrinkage. In general, this fixative can rapidly penetrate to provide well preservation of the tissues. The yellow staining enables the tissues to be seen more easily in embedding step. But Bouin causes partial lysis of red blood cell, and may result in swelling of collagen fiber.

Formaldehyde is a gas which is water soluble extends to the maximum of 40% formalin. This solution is nearly acid and becomes formic acid in stuck solution. The magnesium and calcium carbonate are usually allowed to neutralize the solution. Formalin is diluted with tap water, normal saline or buffer- salt solution, commonly in the proportion of 10 parts of formalin to 90 parts diluent. Therefore in practice, 10 %

formalin in normal saline means 10 parts formalin in 90 parts physiological saline of mean 4% formaldehyde in physiological saline. Formaline fixes protein by forming additive compound with little shrinkage. It provides the staining of acetic structures (nuclei) with basic dyes and diminishes effect structures (cytoplasm) with acid dyes.

Glacial acetic acid, pure acetic is called glacial because it harden at low temperature. 17 °C. It is used in many fixatives to provide the action of other ingredients. It helps to reduce the swelling of collagen fibers and precipitates nucleoprotine.

Formalin 10 % Solution

For gross observation, the 10% formalin was used to preserve the brain tissue. This fixative contains :

Formaldehyde 40 %	10 ml
Distilled water	90 ml

Formaldehyde is a gas which is water soluble extends to the maximum of 40% formalin. Formalin is diluted with tap water or normal saline commonly in the proportion of 10 parts of formalin to 90 parts diluent. Therefore in practice, 10 % formalin in normal saline means 10 parts formalin in 90 parts physiological saline of mean 4% formaldehyde in physiological saline. Formalin fixes protein by forming additive compound with little shrinkage.

APPENDIX IV

Cresyl Fast Violet Stain Paraffin Section

Fixative : Bouin's solution

Section : Paraffin section at 7 μm

Preparation of stain:

Cresyl Fast Violet (Cresyl Violet Acetate)	1 g
Distilled water	100 ml

Method :

1. Dewax section and remove wax with xylene, rinse in second dish of xylene.
2. Rinse in absolute, 95%, 90%, 80%, 70% alcohol and bring to distill water.
3. Cover with filtered cresyl fast violet, stain for 20-30 minutes.
4. Wash in distilled water and place in 70%, 80%, 90% alcohol.
5. Leave in 95 % alcohol until excess stain has been removed.
6. Pass through absolute alcohol into three changes of xylene and mount in Canada balsum.

Results :

• Nissl substance	Deep purple-dark blue
• Nuclei and some cytoplasmic process of neuron	Purple blue
• Backgroud	Colorless

APPENDIX V

Film Processing and Printing for SEM Photograph

Negative Film Processing

1. Load the exposed film in the rack or film holder in the dark room.

2. Wash the film in the cool distilled water (21-22 °C) for 1 min to clear the distilled water prevents excessive softening of the emulsion.

3. Develop with Microdol-x (22 °C) with continuously stirred for 12.5 min to activated silver halide grains to silver metallic under alkaline condition.

4. Wash in the distilled water for 1 min.

5. Place in stop bath (acetic acid) for 1 min to stop the reaction of the developer.

6. After washing in the distilled water for 1 min, the film is immerged in a fixer to convert silver haline into thiocyanate which is water soluble. This step is under acidic condition which will neutralize excess alkaline developer that prevent film fog.

7. Wash in distilled water before placing in hypoclearing agent for 2 min.

8. The well developed film can be visualized under the room light. It is rinsed in the running tap water for 10-15 min before dipping in the water-repellent fluid (photo flo) 1-2 min to prevent water spots.

9. Finally, the film is dried in the film dryer.

Enlargement and Printing

The quality of the negative film should be evaluated prior enlarging. To classified the quality according to the film contrast are three groups. The low contrast negative film requires the high contrast paper (BH paper), while the median to high contrast negative film should require the normal to low contrast paper (BN papers).

The process of enlargement and printing is done under the safe light as follows:

1. Place the negative film on the negative film holder by emulsion down.
2. Turn on the light source of enlarger and open the aperture widely.
3. Adjust the enlarger by either rising up or lowing down for the appropriated size as need. The image is projected on the easel.
4. The sharp grains on the image, seeing with the image focuser, is adjusted by focusing knob.
5. Adjust the easel until the image fills on the paper.
6. Set the appropriated aperture.
7. Turn off the light of the enlarger, and set the proper expoured time.
8. Place the photographic paper in the easel, and turn on the light of the enlarger and allow to expose it for the desired time.
9. Take the exposed paper and submerged in developer (Dextol : water = 1:2).
10. Transfer the developer paper to the stop bath for 20-30 s.

11. Rinse in the running tap water and dip in fixer for 15 min.
12. Wash the photograph in running tap for 30 min to 1 hr.

13. Submerge in a dilute water- repellent agent (Photo flo) for 15 min.

14. Dry with the print dryer, place the emulsion side up against a drying drum so the glossy print is obtain

The scanning electron micrograph is now ready for evaluation.

BIOGRAPHY

NAME	Mr. Ittipon Phoungpetchara
DATE OF BIRTH	16 July 1972
PLACE OF BIRTH	Phitsanulok, Thailand
INSTITUTIONS ATTENDED	Chiang Mai University,
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