

# Hypothalamic vascularization in the common tree shrew (*Tupaia glis*) as revealed by vascular corrosion cast/SEM technique

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**ABSTRACT** The brains from 14 adult common tree shrews (*Tupaia glis*) of both sexes were used for the study of hypothalamic blood supply. It is found that the hypothalamus is supplied by branches of arteries that form the circle of Willis. The preoptic region of the anterior hypothalamus is supplied by branches of anterior cerebral and anterior communicating arteries, while branches of the ophthalmic, anterior communicating and superior hypophysial arteries contribute blood to the optic chiasma. The infundibulum and the median eminence receive arterial supply from the circuminfundibular anastomosis deriving from superior hypophysial, posterior communicating as well as from tiny branches of ophthalmic arteries. The blood supply of the mammillary body comes from the posterior cerebral, posterior communicating and terminal part of basilar arteries. The venous blood from the circuminfundibular area is collected into 15 to 20 hypophysial portal veins flowing towards the par distalis of the pituitary gland. The veins from the tuberal and anterior mammillary regions drain into the premammillary vein while the postmammillary vein receives blood from postmammillary region. The premammillary and postmammillary veins drain into the network of venules forming by anterior cerebral and anterior communicating veins which run across the optic chiasma to join the basal vein on each side of interpeduncular fossa. They finally drain into the cavernous sinus and superior petrosal sinus.

**KEYWORDS:** Diencephalon, Scanning electron microscope, Microvascularization, Vascular cast.

## INTRODUCTION

The hypothalamus is a part of the diencephalon involving with visceral, autonomic and endocrine functions. It lies at the base of the brain and by definition below the level of the thalamus. The tuber cinereum, which is its lower part, gives the attachment to the pituitary stalk.<sup>1</sup> Although the vascular system of the hypothalamus and the pituitary gland, to all intents and purposes, are entirely separated, the functions of these two regions are intimately linked. Thus, various methods have been used to study the blood supply of the two regions together. So far, the blood supply to the hypothalamus and hypophysis as well as their connections have been intensively investigated. The injection of Indian ink or Berlin blue into the blood vessels supplying this

parts was performed, and specimens were studied macroscopically by tracing in thick or thin sections<sup>2</sup>. Donovan<sup>3</sup> studied the blood supply of the pituitary gland by cutting off each source of blood supply and examine the extents of the subsequent areas of necrosis in the gland. In surveying the development of neuroendocrine interrelation, Harris<sup>4</sup> suggested that the brain influences the function of the pituitary gland by observing that coitus induces ovulation in rabbit. Attention has been drawn to the hypophyseal portal vessels by Popa and Fielding, who reported portal circulation from the pituitary to the hypothalamic region.<sup>5</sup> When Wislocki and King<sup>6</sup> studied the pituitary gland of rhesus monkeys in which the blood vessels were injected with Indian ink, they came with the opposite conclusion that the portal vessels drained the blood from the median

eminence to the pituitary gland. The failure to find significant vascular connection between the pituitary stalk and hypothalamus as well as the constant occurrence of lateral veins draining the gland was an important factor in the adoption of this view point.

After pinching the pituitary stalk in the living rabbits for two minutes and rapid fixation of the region in situ with formalin, the portal vessels were dilated below the clamp and closed above it, indicating that portal vessels were enlarged and engorged with blood. In addition, the base of the stalk was completely collapsed at the hypothalamic end after surviving the rabbits for more than 50 days after stalk section.<sup>7</sup> The hypophyseal portal system of blood vessels has been demonstrated to be the functional linkage between the hypothalamus and anterior pituitary in the mouse. A set of primary capillaries in the neurohypophysis, particularly in the median eminence and neural portion of the stalk, is in contact with termination for nerve fibers of the hypothalamo-hypophysial tracts. These capillary plexi connect to the sinusoid or secondary capillary plexi in the anterior pituitary via a series of the portal trunks.<sup>8, 9</sup> Negm<sup>10</sup> has demonstrated that the hypothalamo-hypophysial vessels run from the median eminence of the hypothalamus along the sides of the par tuberalis to the anterior pole of the par distalis. With the injections of colored substances, Dawson<sup>11</sup> could demonstrate the blood vessels supplying the human optic chiasma also proceed to the hypophysis and hypothalamus. By using Indian ink with double perfusion technique, Ambach and Palkovits<sup>12-15</sup> and Ambach et al<sup>16</sup> could demonstrate the arterial supply and venous drainage of rat preoptic region, nucleus supraopticus, anterior hypothalamus, medial hypothalamus and posterior hypothalamus.

As three-dimensional concept of the extent and topography of the hypothalamo-hypophysial vascular system is difficult to obtain from light microscopic examination of serial sections or of injected whole mounts, the scanning electron microscopy (SEM) of vascular corrosion cast has become a useful technique to study vascular pattern in normal and pathological tissues and organs. With this technique, the vascular patterns in the pituitary-median eminence complex have been studied in the rabbit<sup>17</sup> and rat<sup>8, 9</sup>. This technique has also elucidated the vascular pattern in pituitary gland of common tree shrew (*Tupaia glis*)<sup>18</sup>, the animal regarded as a primitive primate.<sup>19, 20</sup> As detail hypothalamic angioarchitecture of the tree shrew has not been

studied, it is of interest to employ the vascular corrosion cast technique/SEM to elucidate this matter.

## MATERIALS AND METHODS

Fourteen adult common tree shrews (*Tupaia glis*) of both sexes were anaesthetized with diethyl ether before the thoracic cavity was opened to expose the heart. Heparin, 0.05 ml (Leo, 5,000 iu/ml) was immediately injected into the left ventricle and allowed to circulate for 1 to 2 min. A blunt no. 18 gauge needle was inserted into the left ventricle and its tip was directed into the lumen of the ascending aorta. A needle was held in place with arterial clamp. The right atrium was cut to open as the outlet port of blood and injected fluid. Approximately 200 ml of 0.9% NaCl solution was infused through the cannula and then the effluent was clear.

The animals were prepared according to the method previously described.<sup>21, 22</sup> The hypothalamic angioarchitecture was then studied by vascular corrosion cast technique combined with stereomicroscopy and scanning electron microscopy (SEM).

## RESULTS

Ventrally, the size of the common tree shrew hypothalamus is 7-7.5 mm in the longitudinal axis and 3-3.5 mm in the transverse axis. It includes the preoptic area, optic tract, optic chiasm, mammillary bodies, tuber cinereum and infundibulum to which the stalk of the hypophysis is attached.

### Arterial supply of the hypothalamus

The arteries supplying the hypothalamus in the tree shrew are all derived from the circle of Willis which is formed by the internal carotid artery (ICA) and the most rostral branches of the basilar artery as well as the anastomotic branches (Fig 1a). It is convenient to separate the hypothalamus arbitrarily into anterior, middle and posterior parts in order to relate to various arterial components of the circle of Willis. The anterior part of the hypothalamus, consisting of the preoptic region, is supplied by branches of the anterior cerebral arteries (ACA) and anterior communicating artery (ACoA) (Figs 1b, 1c). These arteries course dorsally and laterally to enter the brain at nearly equal distance from each other and ramify into many branches to supply the anterior part of the hypothalamus near the midline. The middle part of the hypothalamus is situated caudally

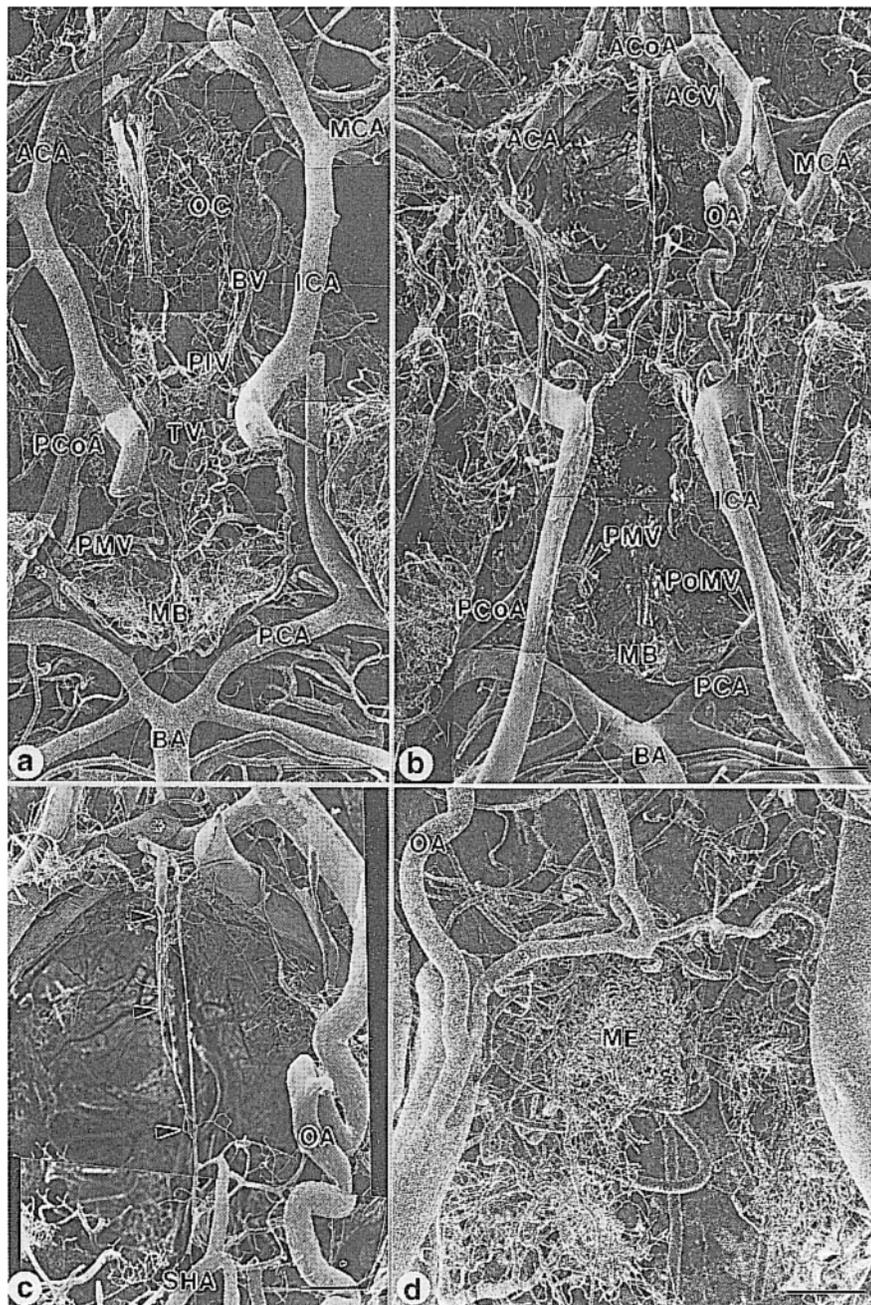


Fig 1. SEM micrographs of the hypothalamic vascular cast

- a. At low magnification, the micrograph shows the blood vessels of hypothalamic region after removing the pituitary gland. ACA, anterior cerebral artery; BA, basilar artery; BV, basal vein; ICA, some part of internal carotid artery; MB, mammillary body; MCA, middle cerebral artery; OC, optic chiasma; PCA, posterior cerebral artery; PCoA, posterior communicating artery; PIV, preinfundibular vein; PMV, premammillary vein; TV, third ventricle; \*, postmammillary vein. Bar = 1 mm.
- b. Another view showing branches of the anterior communicating artery (ACoA) which supplies the preoptic area and optic chiasma (arrowhead). The superior hypophyseal artery (\*) and ophthalmic artery (OA) give branches to supply area of optic chiasma. Note premammillary vein (PMV), postmammillary vein (PoMV) draining the blood from the mammillary body (MB). ACV, anterior cerebral vein; BA, basilar artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PCoA, posterior communicating artery. Bar = 1.5 mm.
- c. Ventral view of common tree shrew hypothalamus vascular cast, at higher magnification, showing that the preoptic area receives blood supply from the anterior communicating artery (\*) and optic chiasma from superior hypophyseal artery (SHA) and ophthalmic artery (OA). Note the anastomosis between the branches of ACoA and that of SHA (arrowheads). Bar = 400  $\mu$ m.
- d. Ventral view of common tree shrew hypothalamus vascular cast, at low magnification, showing that the ophthalmic artery (OA) gives branches to supply the optic chiasma and sends some branches to median eminence (ME). Bar = 400  $\mu$ m.

to the preoptic region beneath and between the proximal parts of the two internal carotid arteries (Fig 1a). It consists of optic chiasma, tuberal region and infundibulum. The optic chiasma is shown to be supplied by the three main sources (Fig 1b).

The first source is from prechiasmal branches of the ophthalmic arteries (OA). They loop along the medial border of the optic nerve and send minute twigs into the parenchyma of the optic nerve (Fig 1d). The second source is from superior chiasmal arteries which are branches of the ACoA. They are small vessels that take origin from the inferior wall of the anterior communicating artery. These arteries pass forward over the dorsal surface of the optic chiasma. They join with tiny prechiasmal branches from the OA. The third source is from the superior hypophysial arteries (SHA) which are branches of the ICA. They arise from the medial aspect of the intracranial segment of the ICA before giving off to the OA. They also give some small branches to join with the prechiasmal branches from the OA and the superior chiasmal branches of the ACoA.

The blood supply of the tuberal region and infundibulum is observed as circuminfundibular anastomosis (Fig 2a) deriving from three main groups of branches from the circle of Willis. They are the SHA from the internal carotid arteries, the infundibular arteries (IA) from the posterior communicating arteries (PCoA) and the tiny branches of ophthalmic arteries.

After arising from the ICA, the SHAs pass medially into the circuminfundibular network (Fig 2a) where they intermingle with infundibular branches from the PCoA and with some branches of the OA. From this conglomeration, the SHAs give major blood supply to the median eminence. The arteries branch off to form two types of capillary plexi, internal and external plexi (Fig 2c). These plexi also communicate with the blood supply of the tuberal region and also or some relatively discrete twigs from the PCoA. In addition, the SHAs send 2 to 3 penetrating branches to join with circuminfundibular anastomosis forming the subependymal capillary network (SC) around the third ventricle (Fig 2b).

Concerning the blood supply of the posterior part of hypothalamus, the mammillary body is one of the most highly vascularized areas of hypothalamus (Fig 3a) and could easily be delineated from its surrounding. Its main blood supply is from branches of the posterior cerebral arteries (PCA) connecting between the basilar artery (BA) and the PCoA. The major parts of mammillary nuclei are supplied by

posterior mammillary arteries (Fig 3b) which are branches from the distal portion of the BA and from the proximal portion of the PCA (Fig 3a). The main blood supply to the lateral part of mammillary nuclei is from the posterolateral mammillary arteries branching from the PCA and the PCoA.

### Venous drainage of the hypothalamus

The network of venules can be observed on the surface of supraoptic region (Fig 1a). It drains the blood into the anterior cerebral vein (ACV) which then courses across the optic tract to join the basal venous circle of the interpeduncular fossa. The basal venous circle, when viewed from the ventral surface, is placed between the brain and the arterial circle of Willis.

From the ventral aspect of the optic chiasma (Fig 1a), the arborization of veins flows into the anterior communicating vein (ACoV) and the ACV by way of two main venous trunks. There are also some communicating venous channels situating near the anterior and lateral chiasmal borders that carry the venous blood into the venous networks in the interpeduncular fossa. Another venous arch called preinfundibular vein (PIV) is also observed immediately anterior to the infundibulum (Fig 2a) between the two veins lining on the lateral borders of optic chiasma (Fig 1a). Its lateral extremity joins the basal vein on each side of interpeduncular fossa.

In the median eminence, the arteries branch off to form two groups of capillary plexi as internal and external plexi (Fig 2c). The internal plexus consists of numerous capillary loops which project into the hypophysial recess of the third ventricle (Fig 2c). The external plexus appears as thin outer shell of capillaries in the median eminence (Fig 2c). Most capillaries in both internal and external plexi drain the venous blood into 15-20 long portal vessels called hypophysial portal veins (Fig 2b). These veins course along the ventral and ventrolateral surfaces of the infundibulum into the pars distalis of the pituitary gland where they branch into sinusoids.

The transverse veins from two sides and the premammillary veins (PMV) (Fig 2a) receive the venous blood from the tuberal region and anterior portion of mammillary nuclei. The postmammillary vein (PoMV) (Fig 3a) occupies the sulcus behind the mammillary bodies. It is joined by tributaries, which originate from blood vessels supplying the bodies and from two sides of them. The premammillary and postmammillary veins empty the blood into the superior petrosal sinus that lies along the ventromedial surface of the temporal lobe. In summary,

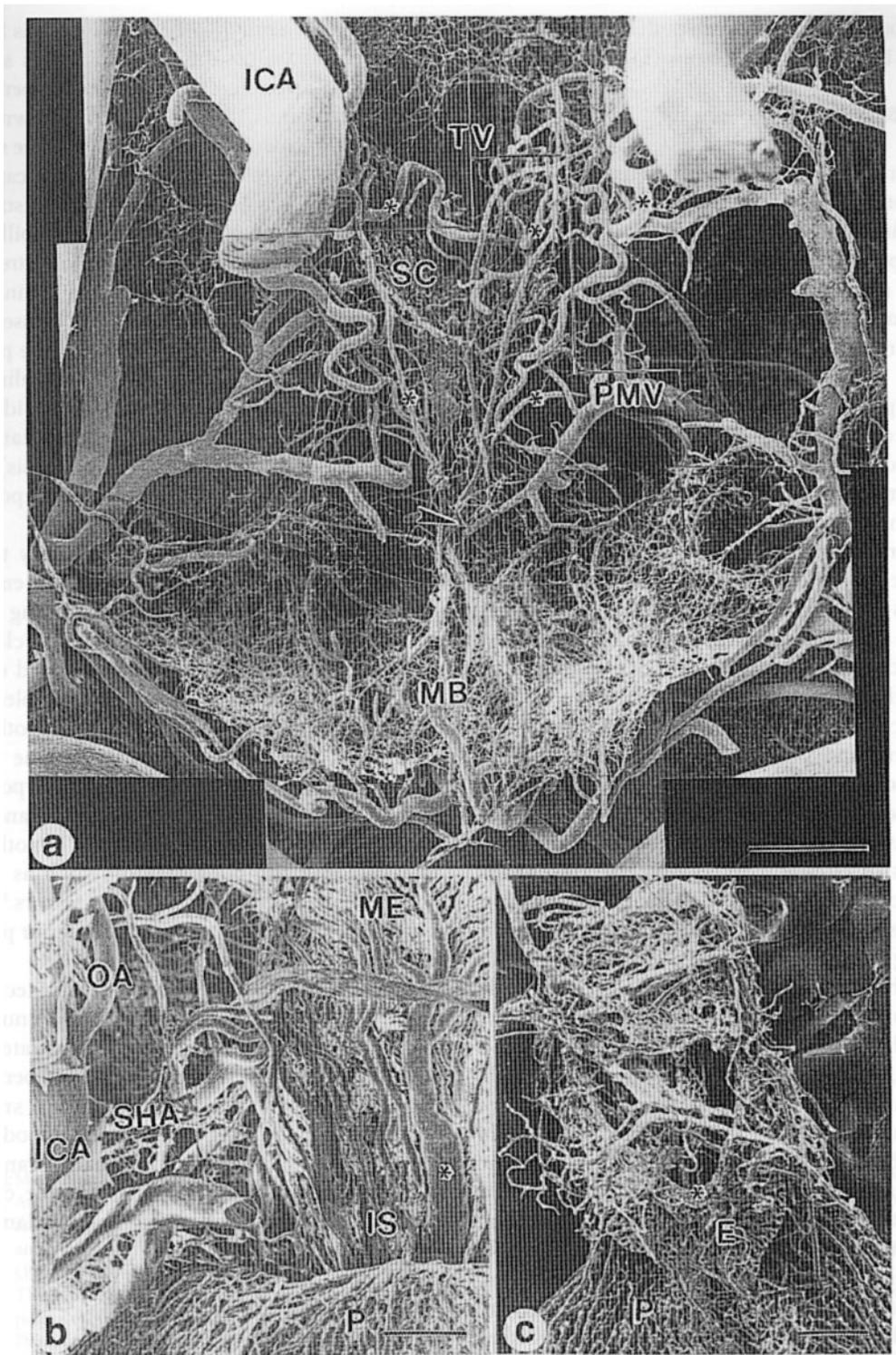


Fig 2. SEM micrographs of vascular cast of blood vessels in the common tree shrew median eminence and mammillary body (MB)  
 a. At low magnification, the micrograph shows the circumfundibular anastomosis (\*) giving their branches to form subependymal capillaries plexus (SC), surrounding the third ventricle (TV). Note the small venules (arrowhead) draining the blood from median eminence and anterior portions of MB to the premamillary vein (PMV). Bar = 400  $\mu$ m.  
 b. Ventral view of common tree shrew median eminence (ME) and infundibulum (IS) showing the superior hypophyseal artery (SHA) which gives branches to this area. ICA, internal carotid artery; \*, hypophyseal portal veins. Bar = 200  $\mu$ m.  
 c. At low magnification, the dorsal view of common tree shrew median eminence vascular cast, showing the external (E) and internal capillary plexus (\*). P, pituitary gland. Bar = 350  $\mu$ m.

the angioarchitecture of the hypothalamus of the tree shrew is illustrated in diagrams (Fig 3c and 3d).

## DISCUSSION

In general, the organization and distribution of the major cerebral arteries in tree shrew are similar to those in human<sup>23, 24</sup> and rat.<sup>10, 12-17</sup> However, there are exceptions as follows. The common tree shrew cerebral arterial circle is formed by the anterior cerebral arteries, posterior communicating arteries, posterior cerebral arteries and internal carotid arteries. The internal carotid arteries become an integral part before bifurcating into anterior and middle cerebral arteries. The findings in the tree shrew are similar to those in the rat but different from human whose arterial circle is formed mainly by the anterior cerebral arteries, posterior cerebral arteries and posterior communicating arteries.<sup>24</sup> The anterior communicating artery is present in the tree shrew as in human<sup>23, 24</sup> but is absent in the rat, opossum and armadillo.<sup>25</sup> The structure in rat that could be compatible with the anterior communicating artery is characterized by the presence of button hole-like structure. In addition, there is corresponding ophthalmic artery in the rat as found in the tree shrew and human<sup>24</sup> as well as in dog and cat.<sup>11, 24</sup>

The sources of blood supply as well as the distributions of arterial and venous blood in hypothalamus of common tree shrew are principally similar to those reported in human<sup>5</sup>, rat,<sup>12-17</sup> dog, cat,<sup>26</sup> opossum and armadillo,<sup>25</sup> even though different studying methods were used. These methods include the intravascular injections with Indian ink, lead chromate, latex and colored gelatin as well as with methyl methacrylate in conjunction with SEM. However, this is different from the case of fish, amphibian, birds and in most of reptiles except for some snakes, of which the internal carotid artery is also the only source of blood supply to the hypothalamus.<sup>27</sup> The transition of the vascular pattern supplying the brain from the primitive form is evident in the amphibian and then the definite mammalian characteristics are observed in reptiles. All the three cerebral arteries (ACA, MCA, PCA) are identified in their evolutionary courses from fishes and amphibians to mammals.<sup>28</sup> As discussed above, the detail information concerning hypothalamic vascularization is lacking. Such discrepancy is elucidated in the tree shrew by the vascular corrosion

cast/SEM technique that the blood vessels forming the circle of Willis and their branches send off penetrating arterioles to supply the deeper part of the hypothalamus. It has also been shown in this study that the median eminence in the tree shrew is nourished by the internal and external capillaries plexi. These vascular plexi are also illustrated in the rat<sup>10, 4</sup>, rabbit<sup>17</sup> and human.<sup>11</sup> The capillaries in this part the hypothalamus of the common tree shrew join together to form 15 to 20 small veins called "hypophysial portal vessels". These vessels carry the hypothalamic venous blood along the pituitary stalk into the anterior lobe or par distalis of the pituitary gland and ramify into sinusoids. The hypophysial portal vessels and the detail blood supply of the pituitary gland (hypophysis) in the common tree shrew have already been reported by Sudwan et al.<sup>18</sup>

It is also demonstrated in this study that the internal capillary plexus of the median eminence forms numerous capillary loops protruding into the infundibular recess of the third ventricle. The subependymal capillary networks located dorsally to these loops are derived from the arterioles which are branches of the hypophysial and hypothalamic arteries. This capillary networks become venules which connect with hypothalamic and hypophysial portal veins. These capillary networks and loops might be necessary for catching the hypothalamic releasing and inhibitory factors such as growth hormone and thyrotropin releasing factors.<sup>8</sup> These factors are known to influence the anterior pituitary functions.

With the vascular corrosion cast/SEM technique, it should be noted that the blood vessels are numerous in the area where the neurons are concentrated when compared to the area occupied by nerve fibers. Even though the hypothalamus is rather small in comparison to the rest of the brain, its blood supply is extremely rich, feeding from many branches of anterior cerebral arteries that forms the circle of Willis. This indicates that the hypothalamus has very high activity.

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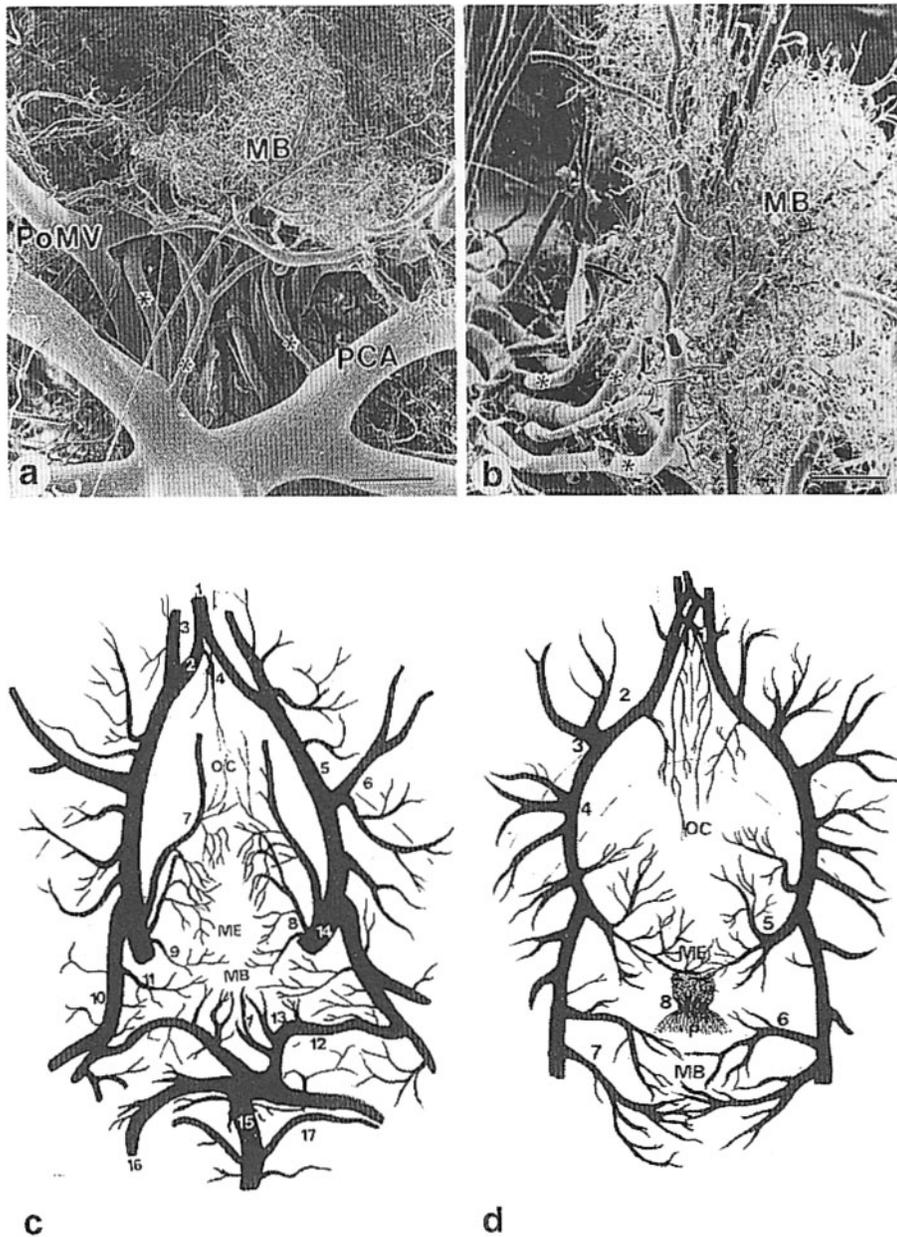


Fig 3. SEM micrographs of vascular cast of blood vessels distributing in the common tree shrew mammillary body (MB)

- a. At low-magnification, the ventral view of the vascular cast shows that the common tree shrew mammillary body (MB) receive blood supply from the posterior mammillary arteries (\*), which are branches of the bifurcation of the basilar artery and proximal portions of posterior cerebral arteries (PCA) and the venous blood is drained into postmammillary vein (PoMV). Bar = 400  $\mu$ m.
- b. The superoanterior view of vascular cast of common tree shrew mammillary body (MB) showing its dense blood supply. \*, posterior mammillary artery. Bar = 250  $\mu$ m.
- c. Diagram of the ventral view of the arterial supply the common tree shrew hypothalamus. 1 = pericallosal artery; 2 = anterior communicating artery; 3 = olfactory artery; 4 = superior chiasmatal artery; 5 = anterior cerebral artery; 6 = middle cerebral artery; 7 = ophthalmic artery; 8 = superior hypophyseal artery; 9 = inferior hypophyseal artery; 10 = posterior communicating artery; 11 = posterior mammillary artery; 12 = posterior cerebral artery; 13 = posterior mammillary artery; 14 = internal carotid artery; 15 = basilar artery; 16 = superior cerebellar artery; 17 = pontine artery; OC, optic chiasma; ME, median eminence; MB, mammillary body.
- d. Diagram of the veins draining blood from the common tree shrew hypothalamus. 1 = anterior communicating vein; 2 = anterior cerebral vein; 3 = middle cerebral vein; 4 = basal vein; 5 = preinfundibular vein; 6 = premammillary vein; 7 = postmammillary vein; 8 = hypophyseal portal vein; ME, median eminence; MB, mammillary body; OC, optic chiasma; P, pituitary gland.

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