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STUDIES ON MORPHOLOGY OF HYPOTHALAMUS IN BACTRIAN CAMELS (*Camelus bactrianus*)

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ABSTRACT

A study on hypothalamus in bactrian camel was done by anatomical, histological and ultrastructural observation and was compared with other artiodactyls. The anatomical results showed that the hypothalamus of bactrian camel can be divided into 3 parts which included the supraoptic, tuberal and mammillary. It was about 2.4 g in weight, 0.42% of the brain and 2 cm^3 in volume and 0.45% of the brain. It had a typical histological structure, which can be divided into 3 zones from the inside out: periventricular, intermediate and lateral zones. The hypothalamus consisted of neurons, neuroglia cells and nerve fibres. Most of the neurons possessed the characteristic of glandular epithelium, in which the neurosecretory granules associated with secretory function can be found by electron microscope. Most of cells in hypothalamus were small neuroglial cells and lateral zone had mainly the longitudinal nerve fibres that were mostly myelinated. Furthermore, the hypothalamus was a brain structure made up of distinct nuclei, as well as less anatomically distinct areas.

Our present result indicated that the typical morphological structure of the hypothalamus and the general level of development of the neocortex gave the bactrian camel moderate ability to process and utilise complex information.

Key words: Bactrian camels, hypothalamus, morphology

The hypothalamus is located below the thalamus, just above the brainstem, forming the bottom wall of the 3rd ventricle (Frandson *et al*, 2003), and is an important central of autonomic nerves (Kent and Van de Graaff, 1995). The hypothalamus is responsible for certain metabolic processes and other activities of the autonomic nervous system (Adamantidis and de Lecea, 2008). It synthesises and secretes certain neurohormones, often called releasing hormones or hypothalamic hormones, and these in turn stimulate or inhibit the secretion of pituitary hormones (Ganten *et al*, 1988; Melmed and Jameson, 2005). The hypothalamus controls body temperature, hunger, important aspects of parenting and attachment behaviours, thirst, fatigue, sleep, and circadian cycles (Hikiji *et al*, 2004; Kim and Lee, 2005; Rocha *et al*, 2004; Meister, 2007). Furthermore, the hypothalamus, mainly the premammillary nucleus, has an important role in expression of innate and conditioned defensive behaviours to a predator (Cezário *et al*, 2008; Ribeiro-Barbosa *et al*, 2005; Blanchard *et al*, 2003). Likewise, the hypothalamus has a role in social defeat (Motta *et al*, 2009).

Hypothalamus of some large artiodactyls, such as horse, buffalo, sheep, pigs and others has also been studied (Nikitenko *et al*, 1970; Jon and Jack, 1975; Jeheskel *et al*, 2006). While studies on the brain and nerves in bactrian camel has also been reported (Cui *et al*, 1998; 2004). Xie *et al* (2006, 2008, 2009, 2011) described the morphology of the brain in bactrian camel by gross anatomy and Nuclear Magnetic Resonance in detail. Chen *et al* (2007, 2008, 2009) compared the near term foetus and adult brain morphology distinction and focused on comparing the difference between the Rhinencephalon and Hippocampus. Bai *et al* (2011) investigated the neurogenesis in dentate gyrus and olfactory bulb of the adult bactrian camel. However, many pertinent characteristics of the hypothalamus in bactrian camel still lack description. The present study was done on hypothalamus from healthy adult bactrian camels by anatomic, histological and electron microscopy techniques, and comparison was made with other artiodactyls.

Materials and Methods

Materials

Ten specimens of the adult bactrian camels were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia

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Autonomous Region, China. Four specimens were removed intact and rapidly, then separated the different parts of the hypothalamus and soaked in 3% glutaraldehyde solution, 10% neutral formalin solution, and 95% alcohol fixative respectively for microscopic and ultrastructural study.

The remaining specimens of the head of bactrian camels were fixed at 5 to 15 min after death. Both internal carotid arteries were isolated by blunt dissection. Cannula coupled by surgical tubing to a pressure-driven perfusion device were inserted into each vessel. Pressure was maintained at a constant 130 mm/Hg for both the initial rinse solution, consisting of 0.5 L of 0.85% sodium chloride, 0.1% sodium nitrite and 0.1% sodium heparin and the fixative which consisted of 3 L of 0.1M phosphate buffered 10% formalin (pH 7.4).

Gross anatomy

The brains were removed from the cranium 1 week after death. The left and right hypothalamus were separated from hemisphere, kept intact and weighed. It was measured by vernier caliper and photographed by digital camera. Data obtained were analysed using the SPSS version 11.5.

Microstructure

Samples for light microscope (LM) were fixed in 10% formaldehyde for 72 h, dehydrated, cleared and embedded in paraffin. Embedded tissues were cut into 7-µm thick sections and stained with haematoxylin and eosin (HE). All the sections were photographed and analysed by MOTIC Images Advanced 3.0 software.

Ultrastructure

Small pieces of the tissue samples for Transmission Electron Microscope (TEM) were pre-fixed in 3% glutaraldehyde buffer (pH=7.2) and fixed for 1 week. The tissues were then washed thrice in 0.1 M phosphate buffer for 30 min before being cut into 1 mm^3 pieces and post-fixed with osmium tetroxide for 1 h. The samples were washed thrice in 0.1 M phosphate buffer and then dehydrated in ascending grades of ethanol before being embedded in epon 812. Semi-thin sections of each tissue were collected and stained with toluidine blue. Ultra-thin sections were then collected on copper grids. The ultra-thin sections were stained with a saturated solution of uranyl acetate for 30 min, followed by lead citrate for 7 min in a carbon dioxide-free environment. Sections were then washed in CO_2-free water, dried and examined under a transmission electron microscope (JEOL, JEM-1230).

Results

Anatomic results

The hypothalamus in bactrian camel were bilaterally symmetrical, it was a region of the brain located below the thalamus, forming the major portion of the ventral region of the diencephalon and the floor of the 3rd ventricle. Landmarks that were visible on the ventral and medial surfaces of the brain defined the boundaries of the hypothalamus. The anterior boundary visible on the ventral surface of the brain was formed by the optic chiasm while the mammillary bodies define the posterior boundary. Most of the hypothalamus was buried in the deep of thalamencephalon, also visible on the medial surface of the brain was the hypothalamic sulcus, which was the anterior continuation of the sulcus limitans that defines the superior boundary of the hypothalamus; the anterior and lateral boundary of the hypothalamus that were surrounded by the olfactory region and the subthalamus of the brain; the anterior boundary that were not visible including the lamina terminalis and the anterior commissure, also the posterior boundary that were not visible include the central gray and tegmentum of mesencephalon, only the ventral surface was free. Optic chiasm, infundibular stalk, mammillary body as landmarks were visible on ventral surface, which divided the hypothalamus into 3 parts, as the supraoptic part that located above the optic chiasm, tuberal part located above the infundibular stalk and mammillary that includes a mammillary body and the posterior region of the hypothalamus. The mammillary bodies of the hypothalamus in bactrian camel were not obvious on ventral surface, which was only a tiny protrusion, but it was clearly observed on the median sagittal plane (Fig 1).

The hypothalamus was separated from brain, kept intact, weighed and measured the parameters of anatomical parts by vernier caliper. The hypothalamus was about 1.55 ± 0.18 (cm) in bottom breadth; 1.72 ± 0.21 (cm) in length; 1.17 ± 0.25 (cm) in height (from the pituitary stalk to the hypothalamic sulcus), while the height of the anterior hypothalamus was about 0.5 ± 0.07 (cm) and 0.81 ± 0.07 (cm) of the posterior hypothalamus. Observation from the median sagittal plane, mammillary body was oval, 0.43 ± 0.08 (cm) in length and 0.37 ± 0.03 (cm) in breadth. The hypothalamus of bactrian camel was about 2.4 ± 0.34 (g) in weight, the percentage of the hypothalamus weight / brain weight was 0.42% (brain weight was 568.3 ± 63.48 g) (Xie *et al,* 2006). The volume was about 2 ± 0.28 (cm^3), the percentage was 0.45% (brain volume was 446 ± 0.18 cm3) (Xie *et al,* 2006) (Table 1).

LM results

Most of the hypothalamus of bactrian camel was buried in the deep of thalamencephalon, only the ventral surface was free. On the ventral surface of the hypothalamus was covered with a thin layer of dense connective tissue capsule known as pia mater (PM). The pia mater of bactrian camel was relatively developed and consisted mainly of densely packed bundles of elastic fibrils which were more tightly packed at the surface of the capsule, as well as infrequently collagen fibres located in the inner layer and connected to the brain parenchyma (Figs 2a, b).

The parenchyma of the hypothalamus was mainly comprised of neurons, neuroglial cells and nerve fibres. The most of nuclei in the hypothalamus of bactrian camel had ill-defined landmarks, and were difficult to distinguish. It appeared that neurons in an area that was densely distributed could be as nuclei, and neurons distributed loosely area could be as the area between the nuclei. The neurons in hypothalamus of bactrian camel were characterised by their larger size, round or oval nuclear, and some of which were accompanied by the clear axons. The most of cells in hypothalamus were neuroglial cells that were smaller generally with round, or oval nuclear (Fig 2c). While it was difficult to distinguish the different types of the glial cells by light microscope. In addition, there were more abundant blood vessels in the parenchyma (Fig 2d).

Table 1. Morphometrics and analysis of the hypothalamus in bactrian camel.

Items	N	Mean	SD	Min	Max	Range
Weight (g)	6	2.40	0.34	2.01	2.73	0.72
Volume (cm^3)	6	2.00	0.28	1.60	2.30	0.90
Length (cm)	6	1.72	0.21	1.56	1.94	0.38
Highness (cm)	6	1.17	0.25	0.84	1.25	0.41
Highness of anterior hypothalamus (cm)	6	0.50	0.07	0.41	0.67	0.26
Highness of posterior hypothalamus (cm)	6	0.81	0.07	0.62	0.96	0.34
Breadth (cm)	6	1.55	0.18	1.31	1.76	0.45
Length of mammillary body (cm)	6	0.43	0.08	0.35	0.51	0.16
Breadth of mammillary body (cm)	6	0.37	0.03	0.32	0.42	0.10

The hypothalamus of bactrian camel was composed of 3 longitudinally oriented cell columns, or zones:

Periventricular zone (PZ): Immediately bordering the 3rd ventricle, just inside of the ependymal cell lining was a thin layer of cells that comprised the periventricular zone. The ependymal cells were composed of columnar cells, palisading closely, elongated rectangular or oval nuclei in the base of the cell, and colour deep. And there were microvilli on the free surface of the ependymal cells. Periventricular zone contained scattered neurons and an amount of neuroglial cells (Fig 3a).

Intermediate zone (IZ): This zone displays several cyto-architectonically distinct nuclei, and was mainly composed of uneven density, ranging in size of neurons and a small amount of neuroglial cells. Neurons in an area that were densely distributed can be considered as a nuclei and neurons distributed loosely area were considered as the area between the nuclei (Figs 3b, c).

Lateral zone (LZ): This zone had few nuclei or clear landmarks, while it contained important fibre pathways such as the median forebrain bundle, and a lot of glial cells that scattered among the nerve fibres. The neurons of this zone had large size, round, or oval nuclear, and the clear axons (Figs 3d-f).

TEM results

The hypothalamus of bactrian camel was mainly comprised of neurons, neuroglial cells and nerve fibres. Neurons in the hypothalamus were characterised by their larger size, with protrusions and low electron density. According to the presence or absence of cytoplasmic secretory granules, the hypothalamic neurons can be divided into: secretory neurons and non-secreting neurons. The secretory neuron was characterised by the presence of different-size round secretory granules, typically electron dense and discrete, and the cytoplasm was usually abundant in cellular organelles with mitochondria, rough endoplasmic reticulum and ribosome. The mitochondria were round with bubble-shaped crest, and rough endoplasmic reticulum were more developed and spread throughout the cytoplasm (Fig 4a). The neurons that had no secretory granules within the cytoplasm were non-secreting neurons. The non-secreting neurons possessed the clear nucleoli in their nucleus, and the mitochondria were round, crest bubbly or dense. Like in the secretory neuron, rough endoplasmic reticulum, ribosomes and Golgi were more developed in the non-secreting neurons.

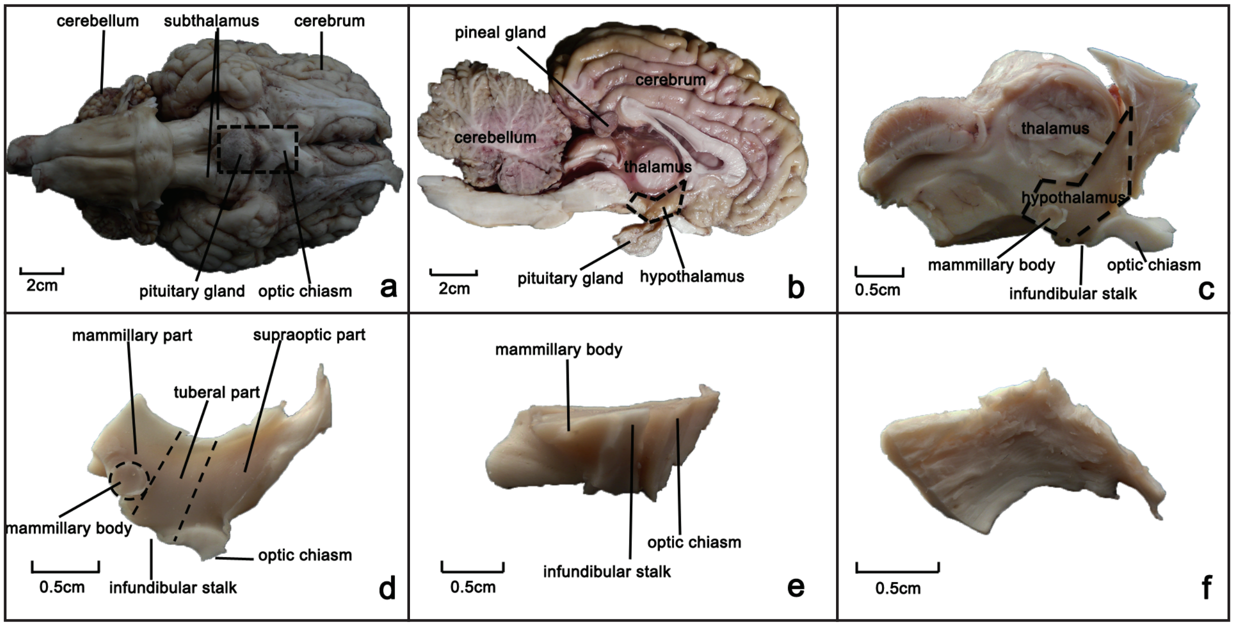

Fig 1. Gross anatomy of the hypothalamus in bactrian camel. a and b, Landmarks defined the boundaries of the hypothalamus were visible on the ventral and medial surfaces of the brain, respectively. The black dotted lines box indicated the hypothalamus region. Bar. 2cm; c, The left hypothalamus and thalamus were separated from hemisphere. The black dotted lines box showed the hypothalamus region. Optic chiasm, infundibular stalk, mammillary body as landmarks were visible on medial surface, Bar. 0.5cm; d, The left hypothalamus was separated from hemisphere. The black dotted lines showed the 3 parts of hypothalamus, as the supraoptic part that located above the optic chiasm, tuberal part located above the infundibular stalk and mammillary that included a mammillary body and the posterior region of the hypothalamus. Bar. 0.5cm; e, The ventral surfaces of the left hypothalamus. Bar. 0.5cm; f, The facies medialis of the left hypothalamus. Bar. 0.5cm.

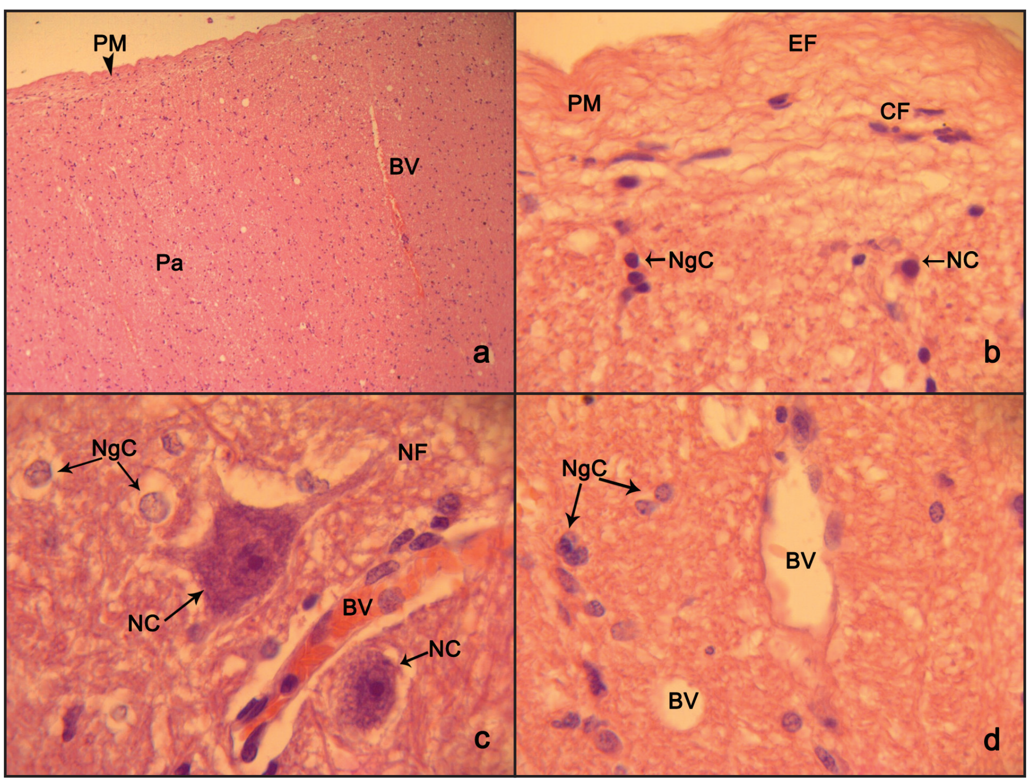

Fig 2. Light micrograph of the hypothalamus in the bactrian camel. a, Hypothalamus: pia mater (PM), blood vessel (BV), parenchyma (Pa). H&E, ×100; b, Pia mater: pia mater (PM), collagen fibrils (CF), elastic fibres (EF), neuron cells (NC), neuroglia cell (NgC). H&E, ×1000; c, Parenchyma: neuron cells (NC), neuroglia cell (NgC), blood vessel (BV), nerve fibres (NF). H&E, ×1000; d, Blood vessel: blood vessel (BV), neuroglia cell (NgC). H&E, ×1000.

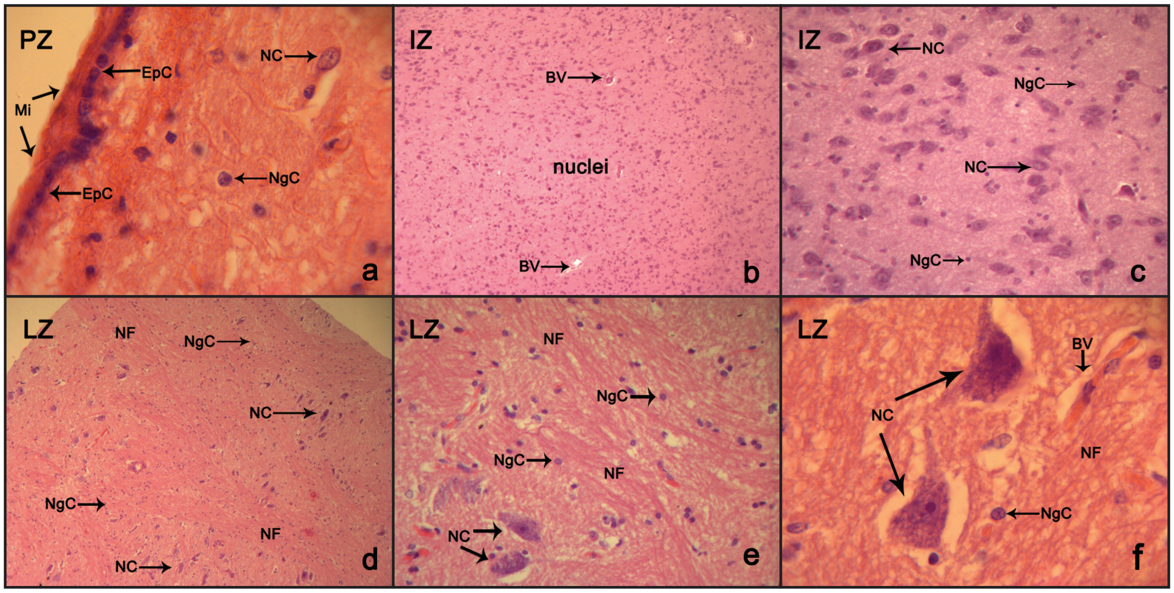

Fig 3. Light micrograph of the 3 zones in hypothalamus. a, Periventricular zone: periventricular zone (PZ), ependymal cell (EpC), microvilli (Mi), neuron cells (NC), neuroglia cell (NgC). H&E, ×1000; b-c, intermediate zone: intermediate zone (IZ), blood vessel (BV), neuron cells (NC), neuroglia cell (NgC). H&E, (b, ×100; c, ×400); d-f, Lateral zone: lateral zone (LZ), neuron cells (NC), neuroglia cell (NgC), nerve fibres (NF), blood vessel (BV). H&E, (d, ×100; e, ×400; f, ×1000).

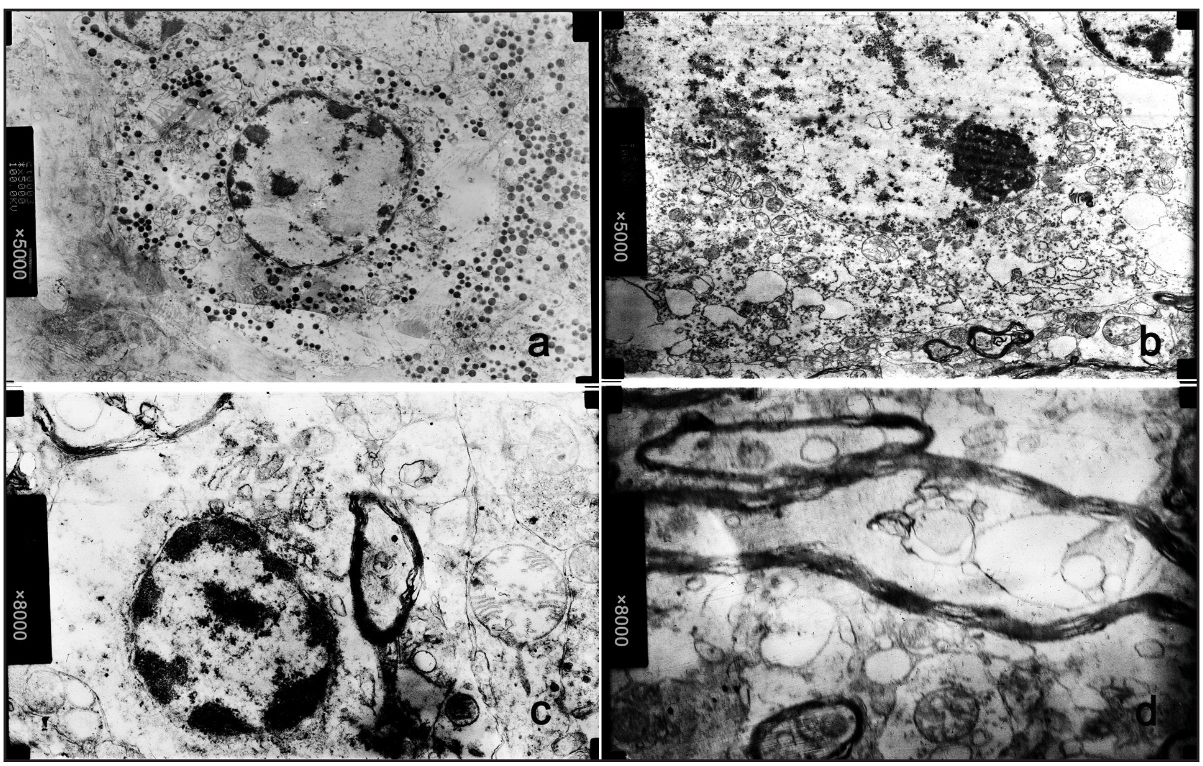

Fig 4. Ultrastructure of the hypothalamus in the bactrian camel. a, Secretory neuron. TEM, ×5000; b, Non-secretory neuron. TEM, ×5000; c, Neuroglia cell. TEM, ×8000; d, Myelinated nerve fibre. TEM, ×8000.

Meanwhile, lysosomes were also more abundant, located in the periphery of the nucleus (Fig 4b). The neuroglial cells in hypothalamic had varied shapes, smaller size, little cytoplasm, generally circular nucleus, and dense electron density. The neuroglial cells were poor in cellular organelles, only some mitochondria with irregularly shaped and tubular cristae and some of the rough endoplasmic reticulum with more free ribosomes can be clearly observed (Fig 4c). The nerve fibres in hypothalamus were mostly myelinated nerve fibres that the centre is axon, within axons were the mitochondria and neurofibrillary, and the outside of the axons were myelin that was characterised by uniform electron density (Fig 4d).

Discussion

The hypothalamus in bactrian camel has a more typical morphological structure. Landmarks that are visible on the ventral and medial surfaces of the brain define the boundaries of the hypothalamus. Optic chiasm, infundibular stalk, mammillary body as landmarks can be visible on ventral surface, although the mammillary bodies are not obvious on ventral surface just like a tiny protrusion, it can be clearly observed on the median sagittal plane. Thus, the hypothalamus can be divided into 3 parts, as the supraoptic part that located above the optic chiasm, tuberal part that located above the infundibular stalk and mammillary that includes a mammillary body and the posterior region of the hypothalamus.

The hypothalamus of bactrian camel was about 2.4 g in weight, the percentage of the hypothalamus weight in the whole brain weight was 0.42%. However, the hypothalamus of human was about 4 g in weight, the percentage of the hypothalamus weight/brain weight was just 0.3%. So the percentage of the hypothalamus weight/brain weight in bactrian camel are larger than that in human, which indicated that the human cerebral cortex is more developed, on the other hand, that the hypothalamus may play a more important role in bactrian camel.

During the development of the brain, the hypothalamus is the more ancient part which developed earlier and later development of the neocortex partially covering wrapped thalamus, Generally, according to the level of development of the neocortex, an animal's level of development of the brain can be assessed. The ratio of the length of the hypothalamus and forebrain can determine the degree of concentration of the brain is called Pilleri index (Pilleri, 1961). In population ecology, the higher pilleri index means more developed neocortex in K selected species, which are characterised by large body size, long life expectancy, low birth rate, low offspring mortality, a better mechanism to protect future generations, and good adaptability to stable habitats. Compared with other artiodactyls, the Pilleri index of bactrian camel is not high, so we considered that a general level of the development of the neocortex in bactrian camel (Nikitenko *et al,* 1970; Jon and Jack, 1975) (Table 2).

Table 2. Some morphometric characteristics of hypothalamus in artiodactyls.

Artiodactyls	Length (cm)	Width (cm)	Length of hypothalamus/ Length of forebrain (Pilleri index)
Pig	1.79	1.20	0.21
Domestic Sheep	2.04	1.07	0.24
Bighorn Sheep	1.65	1.13	0.22
Reindeer	1.5	2.10	0.17
European Elk	1.85	2.80	0.18
Great Horned Cattle	2.21	1.64	0.19
European Wisent	2.09	2.13	0.17
American Plains Buffalo	2.00	1.78	0.178
Bactrian Camel	1.72	1.55	0.153

The EQ of bactrian camel is about 1.11 (Xie *et al,* 2008), which is close to European cat, and only lowers than the gorilla (1.402 to 1.68) (Jerison, 1973; Eisenberg and Wilson, 1981; Jeheskel *et al,* 2006). Furthermore, we found that the Pilleri index is 0.153, which indicates that the bactrian camel has a moderate degree of ability to process and utilise complex information. In conclusion, these hypothalamic structures of bactrian camel are the outcome of a long-term evolution in adapting to a particular environmental niche.

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