



## Ultrastructural Analysis of the vascular zones of the Dromedary Neurohypophysis

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### ABSTRACT

The structural elements of the vascular zones are endothelial cells, basal lamina, pericytes and neuroglial processes that can undergo structural remodelling according to hormonal demand. The qualitative and the quantitative ultrastructural characteristics and seasonal variations of these elements in the neurohypophysis of the dromedary were investigated. The results showed a complex basal lamina, abundant fibrillary elements at pericytes and an important membrane exchange in endothelial cells. Quantitative analysis of neurovascular contacts did not show between seasons, a significant variation in the number of nerve terminals contacting basal lamina, however the mean number of glial processes significantly increased in winter ( $p = 0.037$ ). We think that, as the animal is facing situations of combined stresses in summer, high temperature, insolation..., that lead to increase water demand, these would exert ultra-structural effect on the liberation of neuropeptides at perivascular to decrease water output.

**Keywords:** Dromedary; neurohypophysis; vascular zone; ultrastructure; neurosecretion; water homeostasis

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### INTRODUCTION

The neurohypophysis (NH) is a part of the hypothalamo neurohypophysial (HNS) system that is implicated in the central regulation of water balance. The NH is the place of storage and release of anti-diuritic neuropeptide vasopressin (VP). VP exerts its effect on kidney by retaining water to correct in part dehydration of the organism. The HNS is stimulated to synthesize, store and release of VP according to

certain conditions. Over several past years, researchers have investigated the HNS in mammals under acute or chronic stimuli that induce dehydration or increase osmolarity (Miyata *et al.*, 2001; Gross *et al.*, 1986). It was established then, that the HNS is a dynamic system; its elements undergo important morphological plasticity in response to stimuli, and are reversible after interruption of stimulation

(Miyata and Hatton, 2002). The main elements of neurohypophysal perivascular zones are endothelial cells of blood capillary, pericytes, basal lamina, axonal endings of HNS magnocellular neurons and perivascular glial processes. The aim of this study is to describe and to compare between seasons, the ultrastructural organization of the perivascular zones in an adapted mammal to harsh environment conditions the dromedary *Camelus dromedarius*.

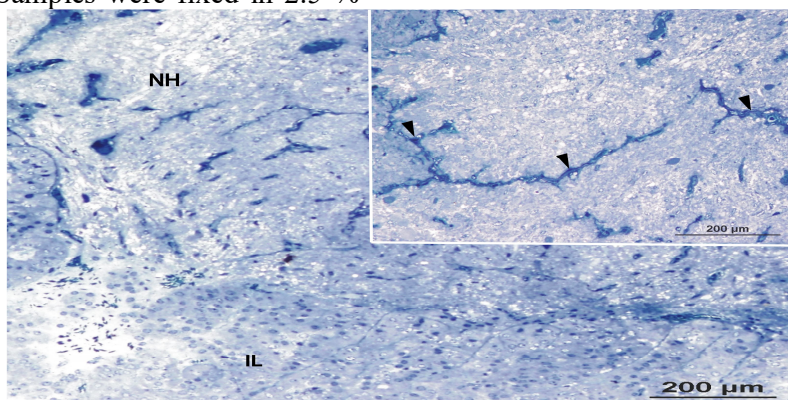
#### MATERIAL AND METHODS

To perform electron microscopic analysis, neurohypophysial lobes of adult male camels (*Camelus dromedarius*) (n=6) were collected in the Algerian Sahara in both winter and summer seasons. Samples were fixed in 2.5 %

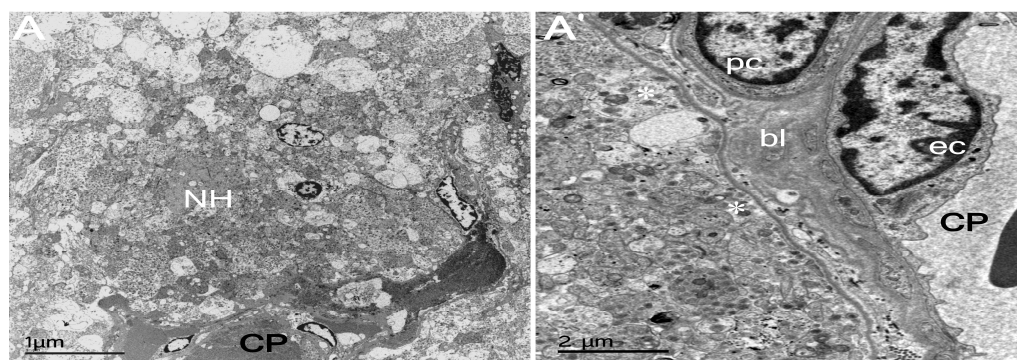
glutaraldehyde in buffered phosphate (0.1 M, pH 7.4) at 4°C. Semithin sections of 1 µm thick were stained with 1% toluidine blue. Ultrathin sections after post fixation in 2% osmium tetroxid and dehydration in graded ethanol and propylene were embedded in spurr's resin. Finally, they were double stained with uranyl acetate and lead citrate; observations were performed using the electron microscope TEM (JEOL 1010).

#### RESULTS

The neurohypophysis (NH) of dromedary under light microscopy showed a rich vascularisation (Figure 1). At ultrastructural level, these perivascular zones seem to be very complex and irregular in shape (Figure 2A).



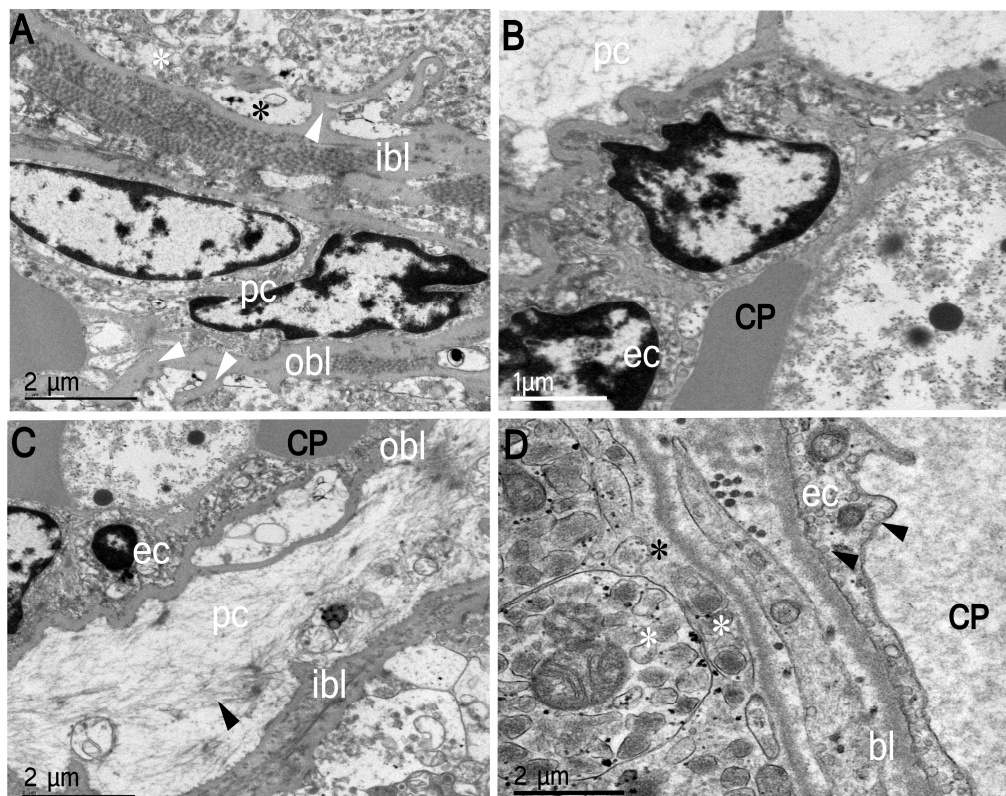
**Figure 1:** Semi-thin section of neurohypophysis, demonstrating vascular and perivascular zones (dark blue) magnified at right (arrowheads). NH: neurohypophysis; IL: intermediate lobe. Toluidine blue. The main elements are: neuronal end feet, basal lamina (bl), pericytes (pc) and endothelial cells (ec) (Figure 2A').



**Figure 2.** A, A' Electron micrographs demonstrating the ultrastructural elements of vascular and perivascular zones. bl: basal lamina; CP: capillary; ec: endothelial cell; pc: pericyte; asterisks: neuronal profiles (axon terminals).

The bl is very complex, it is composed of an inner and outer slices; the space between them is wide where the pc can be found. The bl also showed several protrusions towards NH parenchyma (Figures 3A, B). The

pc frequently contains fibrillary elements (Figures 3B, C). Moreover, an important exchange is observed at the level of endothelial cells, this is reflected by membrane invaginations at luminal and abluminal sides of these cells.



**Figure 3:** Electron micrographs showing neuroglial elements of NH parenchyma in perivascular zones and resident cells of vessels. Note that neuroglial profiles are separated from the capillary by the basal lamina and the pericyte is placed between the inner and the outer slices of the bl CP: capillary; ec: endothelial cell; pc: pericyte; ibl: inner basal lamina; obl: outer basal lamina; Dark asterisks: glial processes, light asterisks: axonal terminals; (dark arrowheads): membranous structures. (Light arrowheads): basal lamina protrusions.

## DISCUSSION

The neurohypophysis (NH) is a circumventricular organ (Kaur and Ling, 2017), its blood system is outside blood brain barrier (BBB) (Broadwell *et al.*, 1983). This is to increase permeability and allow rapid diffusion of neurosecretory peptides into blood stream.

However, this system as was observed in our study is complex. The NH parenchyma is abundantly scattered by vessels sets, the basal lamina generally composed of an outer (obl) and an inner (ibl) slices, showing frequent protrusions into parenchyma that would increase level of axonal terminals products release into the blood stream. These



observations are in accordance with what was reported by Nishikawa et al (2017). These authors after chronic rat imbibitions with 2% NaCl have observed an increased numbers of perivascular protrusions and surface area of the perivascular space. The pericytes as were reported by the same authors are found between the inner and outer basal lamina suggesting that these cells are responsible for perivascular space reconstitution. In our work these cells are enriched on fibrillary cytoplasmic structures that would be responsible in varying capillary diameter by a possible contraction and dilatation of capillary. The endothelial cells showed the presence of membranous structures that originate from abluminal plasma membrane and others are found originating from luminal membrane to probably release products towards the lumen of the capillary. The number of pituicyte processes is significantly different between summer and winter, this is not the case of axon terminals abutting the bl. Probably because the summer is a potential stimulator of hormonal release than the winter. In winter the hormonal demand for water regulation is reduced (Djazouli Alim *et al.*, 2012). Therefore, in winter, the pituicytes increase their processes number on the basal lamina to enclose nerve terminals and decrease neuropeptides liberation. The coverage of basal lamina by neurosecretory nerve fiber endings is a plastic phenomenon depending on dehydration state of organism (Hatton, 1988), when hormone demand is important pituicyte processes retract from both areas allowing the neuronal terminals direct access to perivascular space.

It is concluded from this study that, all the elements of perivascular area of dromedary NH, participate in vascular space reconstitutions. It seems that dromedary under stress of water lack and insolation and high temperature of summer have a particular complex organization of vascular zones in order to face efficiently to any situation of extreme dehydration by adapting these zones for a better hormonal output.

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