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Histological And Histochemical Study Of Hypothalamus In Adults New Zealand Rabbits

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ABSTRACT

The hypothalamus is a crucial part of the brain responsible for regulating key physiological functions related to homeostasis and behavior. Due to its significance, it has garnered interest from neuroscientists aiming to better understand how its subregions connect with other parts of the central nervous system. This study utilized ten adult New Zealand rabbits, from which hypothalamic tissue samples were collected. The specimens were treated and stained with Hematoxylin and Eosin (H&E), in addition to a specialized stain for further analysis.

The objective of this investigation was to provide a detailed histological analysis of the hypothalamus and its various nuclei in adult New Zealand rabbits. Tissue samples were prepared for microscopic examination using light microscopy. The findings revealed that the hypothalamus in these rabbits consists of five nuclei: the paraventricular (PVN), anterior (AN), dorsomedial (DN), ventromedial (VN), and posterior (PN) nuclei. The paraventricular nucleus is located bilaterally next to the third ventricle, consisting of neurons of diverse morphologies, organized in clusters and irregular formations interlaced with nerve fibers, neuroglial cells, and blood capillaries. The anterior nucleus, located near the optic chiasma, consisted of predominantly large neurons interwoven with nerve fibers. The dorsomedial and ventromedial nuclei were centrally located within the hypothalamus and contained small, rounded to oval neurons. The posterior nucleus, found near the mammillary body, was primarily composed of large, fusiform neurons.

Keywords: *hypothalamus; histology; Rabbit; hypothalamic nucleus.*

1. INTRODUCTION

Rabbits serve as an important meat source in many countries, reinforcing that they may become increasingly utilized in specific biotechnology projects. They are widely recognized as valuable experimental models in biomedical research (Hussein et al., 2024). The scientific community employs rabbits as test subjects and tools for producing biotechnology products, as well as for breeding purposes (Bõsze and Houdebine, 2006).

The hypothalamus is an important part of the brain that regulates vital physiological processes related to homeostasis and behavior. Due to this role, it has drawn significant interest from neuroscientists aiming to better understand how its subregions interact with other parts of the central nervous system and how these connections influence the organism. Located in the ventral diencephalon, the hypothalamus is a bilateral structure positioned lateral to the third ventricle and above the pituitary gland was bordered laterally by the cerebral peduncle, dorsally by the zona incerta, and transitions caudally into the mesencephalon and periaqueductal gray matter (Simerly, 2015a; Ali and Sami, 2019). Comprising a collection of diffusely arranged nuclei and intricate fiber tracts, the hypothalamus is involved in several specialized functions (AL-Mutar, 2017). It is often referred to as the "master endocrine gland" due to its regulatory influence over the pituitary gland (Krieger, 1971).

(Crosby and Woodburne, 1940) revealed that the periventricular, medial, and lateral longitudinal zones make up the hypothalamus. Superimposed on these zones, the nuclei are further classified into subregions regions: preoptic, anterior, tuberal, and mammillary (Simerly, 2015a).

2. MATERIAL AND METHODE

Ten healthy adult New Zealand rabbits were obtained from local rabbit breeding farms for this study. Instantaneously post-slaughter, the dorsal section of the skull was excised, and the entire head, inclusive of the brain, was submerged in a 30% formalin solution. (AL-Mahdawi et al., 2015; AL-Salihi et al., 1990). The brain was let to solidify in the fixative for several hours prior to meticulous extraction from the skull to reduce tissue damage. The samples of the brain were then sectioned to

expose the hypothalamus. Serial sections, approximately 4-6 µm thick, were prepared and stained using the following methods: Harris Hematoxylin and Eosin (Harris, 1900) for general histological assessment; Grimelius silver impregnation technique for detecting endocrine granules as a non-specific stain for neurosecretory granules(Grimelius, 1968; AL-Salihi et al., 1990; Al-Saffar and Al-Haaikm, 2017) Utilize Performic acid alcian blue stain (PA-AB) (Adams and Sloper, 1955) for the particular identification of neuroendocrine granules. These staining procedures were conducted following the guidelines outlined by (Bancroft & Stevens, 1996; Bancroft and Gamble, 2008).

3. ETHICAL DECLARATION

All experiment were performed in accordance with Iraqi law and the university's protocols for the welfare of experimental animals. The techniques in this study received approval by the Faculty of Veterinary Medicine Committee at Baghdad University, Iraq (PG:2306) on December 3, 2024.

4. RESULTS AND DISCUSSTION

The New Zealand rabbit's hypothalamus was in the most ventral part of the diencephalon. This finding aligns with David L. Clark's (2009) description of the human hypothalamus, located on both sides of the walls of the third ventricle, beneath the hypothalamic sulcus. It is delimited anteriorly by the optic chiasm, laterally by the optic tracts, and posteriorly by the mammillary body. The hypothalamus is situated next to the third ventricle, with the hypothalamic sulcus delineating its superior boundary. (Fig. 1). The ventral region of the hypothalamus is situated at the base of the brain. It is a small, central region consisting of nerve fibers (tracts) and clusters of nuclear bodies, located beneath the thalamus and separated by the hypothalamic sulcus, which runs along the lateral wall of the third ventricle. The anterior part of the hypothalamus, known as the supraoptic area, is defined by a gray matter layer of triangular shape extending above the optic chiasm. Posteriorly, the hypothalamus extends to the tegmentum of the upper part of the brainstem, near the mammillary body, and laterally to the third ventricle. It connects to the thalamus, tegmentum of the midbrain, pituitary, amygdala, hippocampus, and olfactory bulb. (Saper and Lowell , 2014; Paxinos and Watson, 2014) also noted that the human hypothalamus extends to the tegmentum of the brainstem, near the mammillary body, and borders the third ventricle laterally. It consists of several nuclei, including the paraventricular nucleus (PV), anterior nucleus (AN), dorsomedial nucleus (DN), ventromedial nucleus (VN), and posterior nucleus (PN), which is consistent with (Swaab's, 2004) findings in humans.

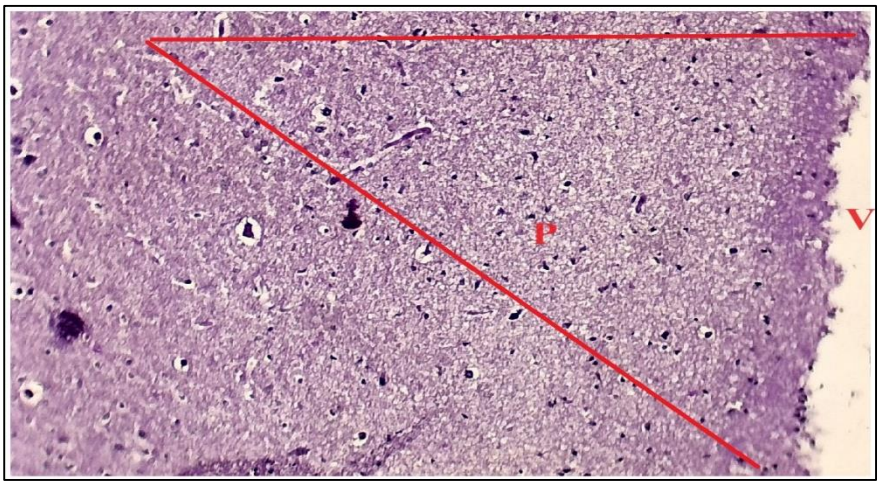

Figure 1: A micrograph of the hypothalamus of the adult New Zealand rabbit show pyramidal shaped organization of PVN (P) around the 3rd ventricle (V). H&E stain, X10.

1. Paraventricular nucleus

The paraventricular nucleus, located in the upper region of the hypothalamus, was made up of neurons with various shapes, including pyriform, triangular, and polyhedral, showing an irregular distribution. These nerve cells were interspersed with fibers and glial cells. The cytoplasm of the neurons contained spherical, intensely basophilic nucleus exhibiting large nucleolus. The cytoplasm itself appeared homogeneous, fine-grained and predominantly basophilic.(Fig.2) These observations are consistent with the findings of (Saper & Lowell , 2014; Paxinos and Watson, 2014). Histochemical analysis showed that the cytoplasmic granules of these neurons stained positively with Alcian blue (Fig.3), suggesting the presence of acidic mucopolysaccharides(Batah and Mirhish, 2022). These results support the established secretory role of PV neurons, especially in the synthesis of vasopressin and oxytocin, as noted by (Sawchenko and Swanson, 1983) in rats.

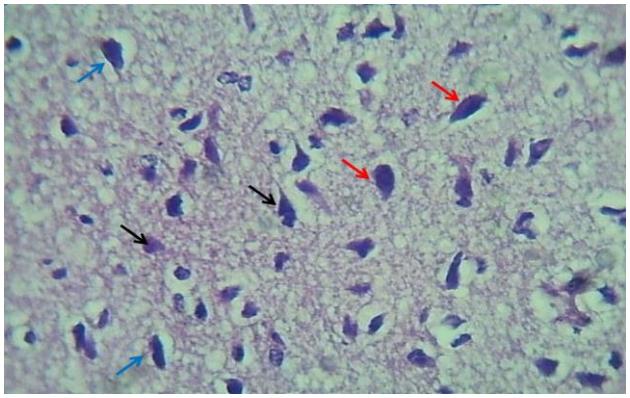

Figure 2: section of hypothalamus- paraventricular nucleus of adults New Zealand rabbits shows polyhedral (blue arrows), pyriform (red arrows)), triangular neurons (black arrows). H&E stain.400x

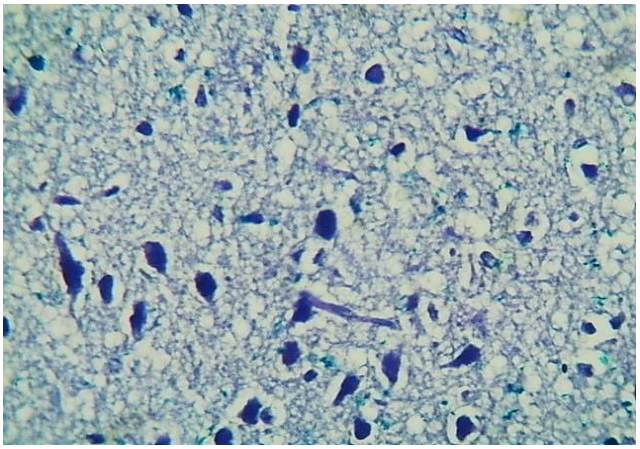

Figure 3: section of hypothalamus- paraventricular nucleus of adults New Zealand rabbits show cytoplasm of neurons contained darkly blue stained granules (Acidic). Combine alcian blue-PAS stain.400x

2. Anterior nucleus (AN)

The nucleus located near the optic chiasma in the supraoptic area was composed mainly of large pyriform neurons interspersed with nerve fibers and glial cells. The cytoplasm of these neurons contained spherical, intensely basophilic nucleus with large nucleolus. The cytoplasm was homogeneous, fine-grained, and generally stained a light basophilic color (Fig. 4). These observations align with the findings of (Junqueira and Carneiro ,2005), who noted that the cytoplasm of the anterior hypothalamic nucleus appeared granular, homogeneous, and lightly basophilic when stained.

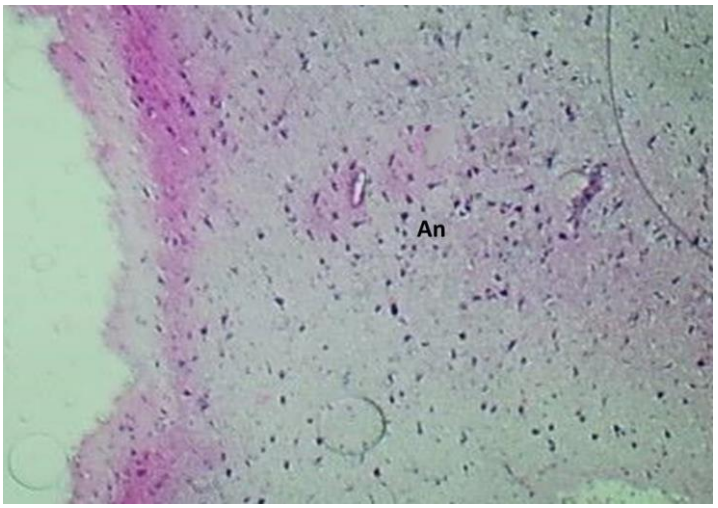

Figure 4: section of hypothalamus- anterior nucleus of adults New Zealand rabbits show (An). H&E stain.100x

When combined with Alcian blue and PAS staining, the neuronal cytoplasmic granules exhibited a strong positive reaction to Alcian blue (Fig. 5). Using silver impregnation staining, the neurons displayed light brownish, argyrophilic granules (Fig. 6).

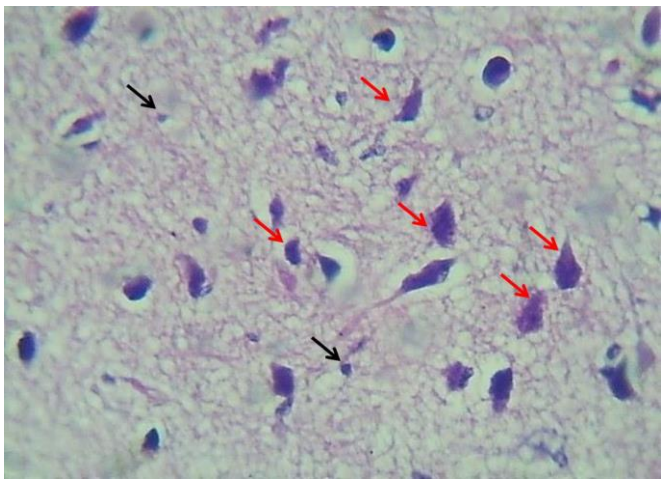

Figure 5: section of hypothalamus- anterior nucleus of adults New Zealand rabbits show large pyriform (red arrows)), glial cells (black arrows). H&E stain.400x

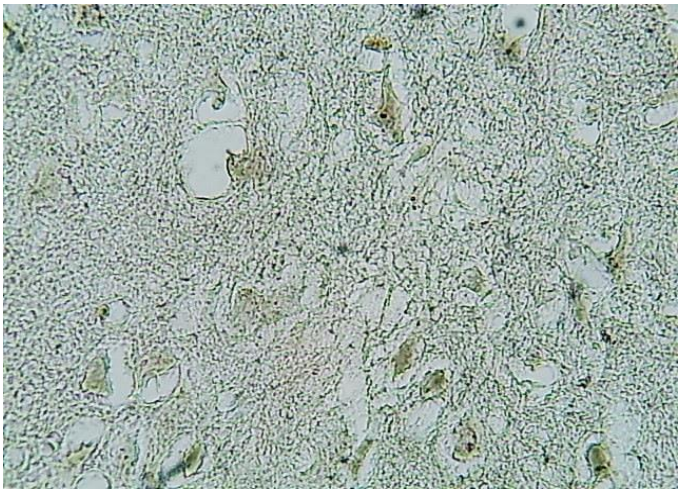

Figure 6: section of hypothalamus- anterior nucleus of adults New Zealand rabbits show the neurons had light brownish argyrophilic granules. Silver impregnation stain stain.400x

3. Dorsomedial & ventromedial nucleuses (DN &VM)

The dorsomedial and ventromedial nuclei were found at the center of the hypothalamus, in an area known as the tuberal region. These nuclei mainly consisted of small, fusiform, rounded to oval neurons that were interspersed with nerve fibers and glial cells, which align with the findings of Swanson (2000). The cytoplasm of these neurons contained intensely basophilic nuclei, with a homogeneous, granular cytoplasm that stained darkly with basophilic dye (Fig. 7 & 8). Histochemical analysis showed strong positive reactions to Alcian blue and PAS staining, suggesting a significant presence of glycosaminoglycans and glycoproteins in the neurons (Fig. 9). This finding is consistent with their role in neurotransmitter and neuropeptide synthesis, as observed in rats by (Sawchenko and Swanson, 1983). Silver impregnation staining revealed dark brownish argyrophilic granules (Fig. 10), which indicate the presence of structural proteins and neurofilaments that support synaptic activity.

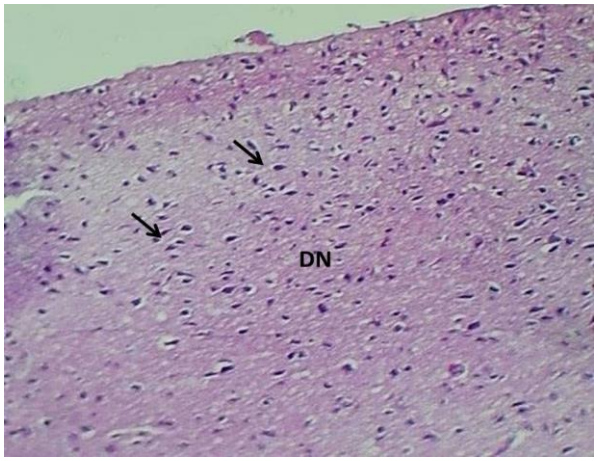

Figure 7: section of hypothalamus- dorsomedial nucleus of adults New Zealand rabbits show almost small size neurons (arrows). H&E stain.100x

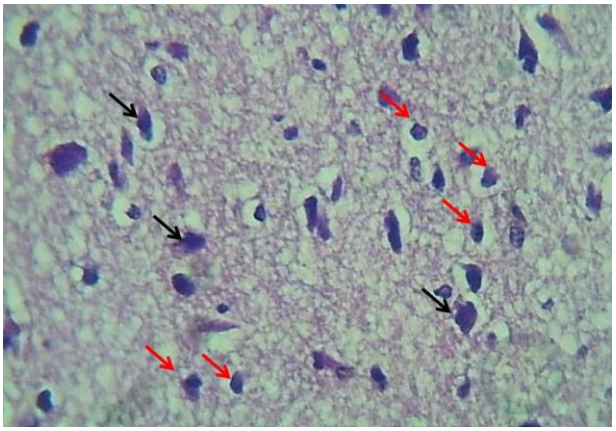

Figure 8: section of hypothalamus-ventromedial nucleus of adults New Zealand rabbits shows small size rounded to oval (red arrows), fusiform neurons (black arrows). H&E stain.400x

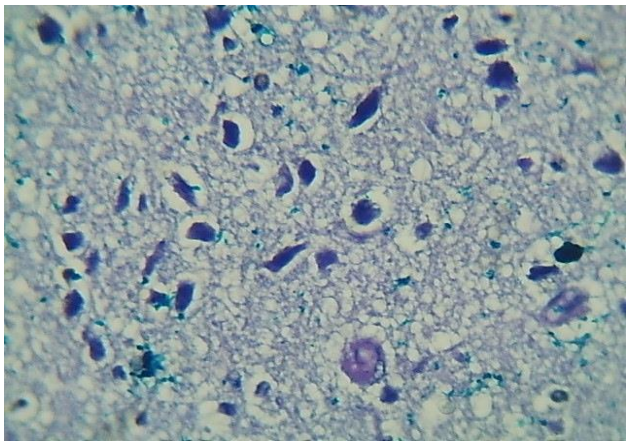

Figure 9: section of hypothalamus- ventromedial nucleus of adults New Zealand rabbits show cytoplasm of neurons contained darkly blue stained granules (Acidic). Combine alcian blue-PAS stain.400x

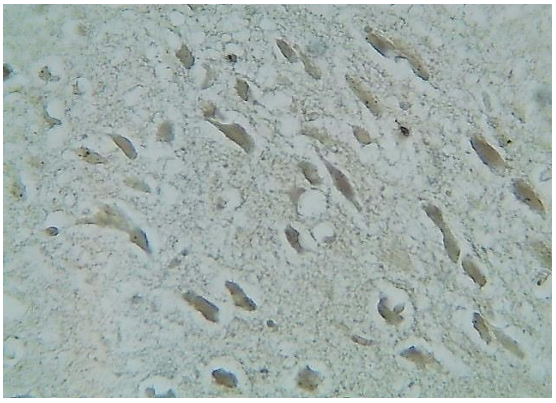

Figure 10: section of hypothalamus- ventromedial nucleus of adults New Zealand rabbits show the neurons had dark brownish argyrophilic granules. Silver impregnation stain stain.400x

4. Posterior nucleus (PN)

The nucleus was located near the mammillary body and contained primarily large fusiform neurons, along with some rounded to oval ones. These neurons were interspersed with nerve fibers and glial cells. The cytoplasm of these neurons featured intensely basophilic nuclei, and the cytoplasm itself was homogeneous, granular, and stained darkly with basophilic dye (Fig. 11). These observations were consistent with the findings of (Junqueira and Carneiro, 2005; Saper & Lowell ,2014). When stained with a combination of alcian blue and PAS, the granules in the cytoplasm of the neurons exhibited a pronounced positive response to alcian blue. (Fig. 12). Additionally, when stained with silver impregnation, the neurons exhibited dark brownish argyrophilic granules. These results align with those of (Burbach et al., 2001; Paxinos and Watson, 2014).

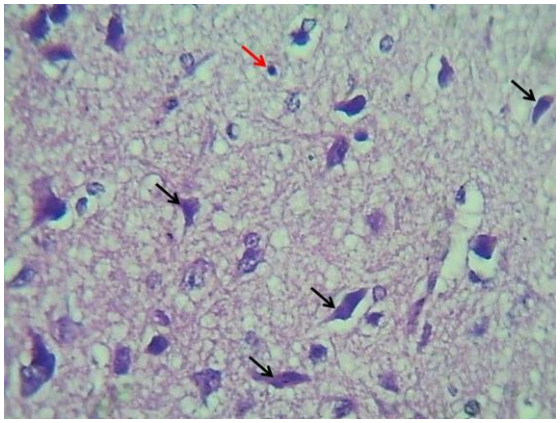

Figure 11: section of hypothalamus-posterior nucleus of adults New Zealand rabbits shows glial cells (red arrows), & numerous fusiform neurons (black arrows). H&E stain.400x

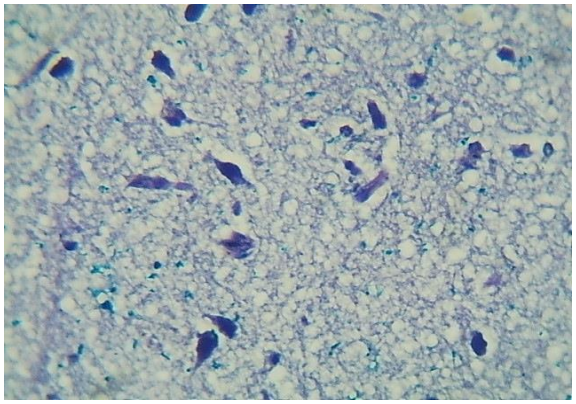

Figure 12: section of hypothalamus- posterior nucleus of adult new Zealand rabbit shows cytoplasm of neurons contained darkly blue stained granules (Acidic). Combine alcian blue-PAS stain.400x

5. CONCLUSIONS

The current study concluded that hypothalamus in rabbits demonstrates a highly organized histological structure with diverse nuclei tailored to specific functional roles. The distinct staining properties and cellular compositions observed in each nucleus highlight their specialization in neuroendocrine regulation, homeostasis, and behavioral responses.

6. RECOMONDATIONS:

The current study recommended Comparative Studies to Future research should examine the structural and functional differences in the hypothalamus across mammalian species to understand evolutionary variations. Also recommended Clinical Implications to study the similarities between rabbit and human hypothalamus suggest that New Zealand rabbits could be useful models for studying neurological and endocrine disorders related to hypothalamic function.

7. ACKNOWLEDGEMENTS

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8. NOVELTY STATEMENT

This study provides a detailed histological examination for hypothalamus in the mature rabbit, offering new insights into the structural organization of its nuclei. The findings align with prior research on human and other animal species, confirming the hypothalamus' position as the most ventral part of the diencephalon and its proximity to key brain structures. Moreover, this research emphasizes the histological features of the paraventricular nucleus, anterior nucleus, dorsomedial and ventromedial nuclei, and posterior nucleus, highlighting their cellular composition, granulation, and neurochemical properties.

9. AUTHORS' CONTRIBUTION

These authors contributed equally.

10. CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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