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Histo-architectural Comparative Analysis of the Hypothalamus of Bat (*Eidolon helvum*) and Wistar Rat (*Rattus norvegicus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SGO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MSM managed the analyses of the study. Author QOM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to compare the histo-architectural variation (if any) on hypothalamus of both Rat (*Rattus norvegicus*) and Bat (*Eidolon helvum*).
Study Design: The histological and histochemical investigations into the hypothalamus of two mammalian species were studied, to determine the possible differences in their thermoregulatory activities.
Methodology: Six (6) rats and six (6) bats were used for this study, the animals were sacrificed under chloroform anaesthesia, after which the skulls of these animals were opened using bone forceps to expose the brains. The hypothalamus were excised from each brain and homogenized to determine the activities of lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), acid phosphatase (ACP) and alkaline phosphatase (ALP). The tissue sample of

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hypothalamus for histological studies were fixed in 10% formol calcium and processed for paraffin wax embedding. Serial sections of 5 µm thickness were stained with Hematoxylin & Eosin, Cresyl fast violet and Gordon & sweet stains. The stained tissues were studied under the light microscope.
Results: The results obtained from the macromorphometric study showed that, there are differences in the body and brain weights of the two mammalian species; bat (275.33±7.49, 3.60±0.09) and rat (193.33±5.30, 2.60±0.08) respectively. The body weight of bat was observed to be higher than that of rat, and the brain weight of each mammal was found to be directly proportional to its body weight. The histological study showed that, neurones are well distributed in rat compared to bat, while the enzyme activity variables of G-6-PD, LDH and ACP were higher in rat with reliable proportion, except ALP that was found higher in bat (1022.00±0.91) compared to rat (829.75±1.31). This suggested that, the hypothalamus of rat performs more functions of thermoregulation, feeding and circadian rhythm control than bat, since the nuclei of hypothalamus such as; lateral hypothalamic nuleus, arcuate nucleus and suprachiasmatic nucleus control feeding, satiety and circadian rhythm respectively, which could be as a result of differences in their modes of behaviour, habitat and feeding.
Conclusion: Considering the histological analysis and the enzyme activity, the hypothalamus of both animals are significantly different, being influenced by feeding and lifestyles.

Keywords: G-6-PD; LDH; ACP; ALP; hypothalamus; neuropil; glial cell; reticulin fiber.

1. INTRODUCTION

Comparative anatomy has long served as evidence for evolution, indicating that various organisms share a common ancestor [1]. Comparing specific organs by relating morphological changes to physiological changes, the relationship between organisms of different species can be determined. The concept of comparative anatomical study is to reveal the differences and similarities in the development, evolution, function and structure of same organ of different species. Thus, comparative Anatomy is very important in the investigation of animals especially living species, in contrast to fossils and also in tracing ancestral lineage of different species of animal [2]. Various researches have been conducted on comparative study of organs, hormone and enzymes in different species and various regions of hypothalamus have been extensively studied using various methods including microdialysis [3,4]. Comparative study of organs in mammals such as rat, bat and pangolin have been carried out by many researchers [5]. The three species of mammals are of interest in comparative study because, they exhibit different modes of behaviour, feeding and habitation.

2. MATERIALS AND METHODS

2.1 Experimental Animals

The Rats were obtained from animal holding of the department of anatomy of the University of Ilorin, acclimatized for forty-eight (48) hours and sacrificed shortly after. The bats were curled down from their roosting colony at the flower garden area of government reservation area (G.R.A.), Ilorin and were treated with tetracycline as prophylaxis against bacterial infection; they were weighed and sacrificed about five hours later. All the animals were weighed and sacrificed during the day on the same day. Six (6) Wistar rats (average 2 months of age) and six (6) bat were used for this study, all animals were males.

2.2 Animal Sacrifice and Tissue Extraction

The experimental animals were sacrificed after being anaesthetized through chloroform inhalation. The organs for histological processing (hypothalamus) were excised with the aid of the atlas of the rat brain as described by Paxinos and Watson [6].

2.3 Histological Staining

Extracted tissues were immediately placed in a specimen bottle containing fixative to prevent autolysis. Part of the tissues were fixed in formol saline for 12 hours, followed by routine histological processing for light microscopy, tissues were embedded in paraffin. About 5 micron-thick serial sections were cut and stained with haematoxylin & eosin [7], cresyl fast violet [8], and Gordon and sweet [9] for the demonstration of the general structure, nissil substances and reticulin fibers resectively.

2.4 Haematoxylin and Eosin Staining [7]

Sections were re-hydrated by first placing in xylene for 5 minutes to dissolve the paraffin wax. They were then passed through;

i. Two changes of descending grades of 90% and 50% of alcohol for 1 minutes each
ii. Washing in running tap water and staining with Haematoxylin for 10 minutes
iii. Differentiation was done in 10% acid alcohol for 4 seconds
iv. Running in tap water for blueing for 5 minutes
v. Counterstaining was done with Eosin for 1 minute
vi. De-hydration through different ascending grades of alcohol starting with 50% alcohol for 2 minutes and changed to 90% alcohol for 2 minutes then two changes of absolute alcohol 1 minute each.

The sections were placed in two changes of xylene for one minute each. Mounting of sections was done using Dimethyl Paraffinate Xylene (DPX), after which the sections were ready for microscopic examination.

2.5 Cresyl Fast Violet Technique – For Nissl [8]

Sections: 5 µm paraffin wax sections

Staining Solution:

- Cresyl Fast Violet- 1 g
- Distilled water- 100 cm^3
- Acetic acid- 0.25 cm^3

Procedure:

- Take sections to the water
- Stain in cresyl fast violet solution for 20-30 minutes
- Leave in 96% alcohol until most of the stain has been removed
- Clear in xylene, and mount sections in DPX
  - Nissl substance-Purple/dark blue
  - Neurone and cell nuclei-Purple/blue

Gordon and Sweet's Method (1936) - Reticular Fibers

Procedure:

- Deparaffinize and hydrate to distilled water.
- Potassium permanganate solution, 5 minutes.
- Wash in water.
- 5% oxalic acid until clear.
- Wash in distilled water.
- Iron alum solution, 10 minutes.
- Wash in running tap water, rinse in distilled, 3 changes.
- Silver solution, 7 dips, shake excess solution off slides.
- Distilled water, 2 changes, 3 quick dips each.
- 10% formaldehyde solution until gray black, 30 seconds.
- Wash in distilled water.
- 0.5% Gold chloride, 1 minute.
- Rinse in distilled water.
- 5% hypo, 1 minute.
- Wash in tap water.
- Nuclear-fast red solution, 5 minutes.
- Wash in running tap water.
- Dehydrate, clear, and coverslip.
  - Results:
  - Reticular fibers black
  - Nuclei red

2.6 Homogenization Procedure

Immediately after, the hypothalamus were weighed and placed in 0.25 M sucrose solution, and homogenized using a homogenizer. The homogenate was centrifuged at 500 rpm for 5 min using a centrifuge (Gallenkomp, England). The supernatants collected, using Pasteur pipettes, were stored at -20℃, and thereafter enzymes study was carried out for the activities of lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), acid phosphatase (ACP) and alkaline phosphatase (ALP). Through spectrophotometry (Colorimetric method), the activities of LDH and (G-6-PDH) were determined in the homogenates using the RANDOX's Kits and the Quimica Clinica Aplicada' skit for ALP.

3. RESULTS

3.1 Macromorphometric Analysis

The body weight and brain weight of both bat and rat are shown in Table 1 using a descriptive statistics.

3.2 Histological Analysis

The photomicrographs of the hypothalamus of both bat and rat at various resolutions of microscopic observation with different stains in Figs. 1-6.

3.3 Analysis of the Activities of Enzymes

The result in Table 2 shows the activities of enzymes in the hypothalamus of both rat and bat.

Table 1. Body and brain weights of bat and rat

Group	Body weight (g)	Brain weight (g)	Brain W./ Body W.
Bat	275.33±7.49	3.60±0.09	0.0131±0.0001
Rat	193.33±5.30	2.60±0.08	0.0135±0.0001

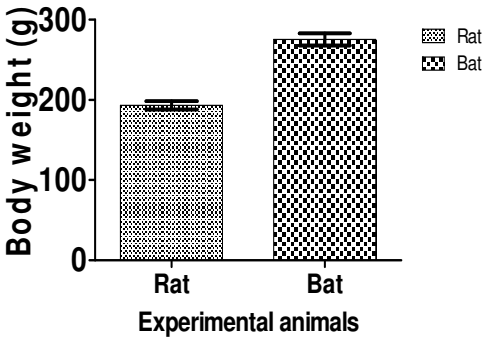

Chart 1. Comparing body weight

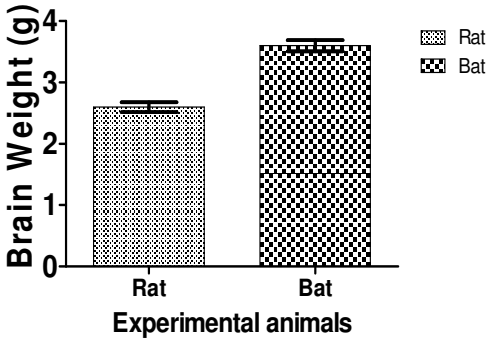

Chart 2. Comparing brain weight

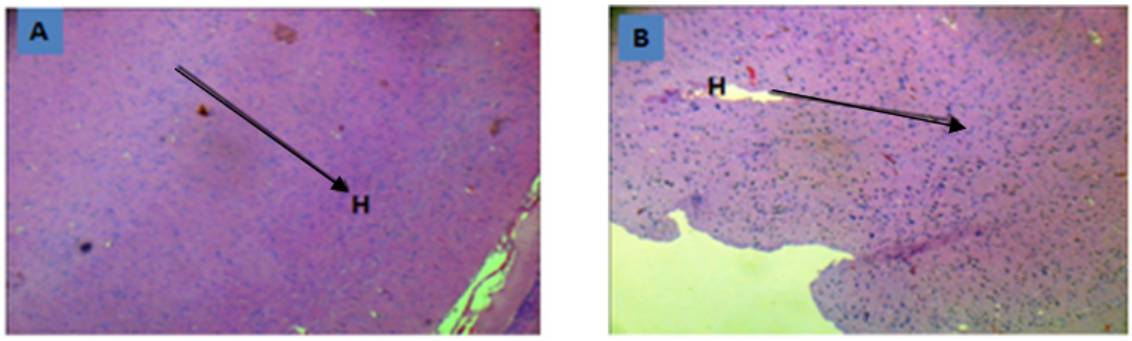

Fig. 1. Hypothalamic section of rat (A) and bat (B) with stained hypothalamus (H). H&E X100

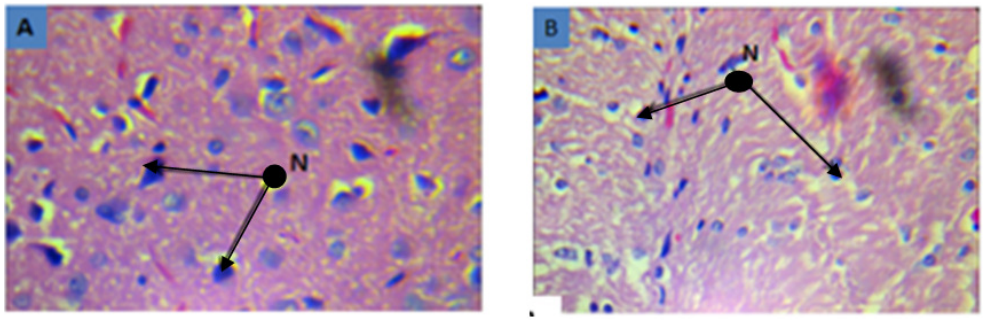

Fig. 2. Hypothalamic section of rat (A) and Bat (B) with stained neuropil (N). H&E X400

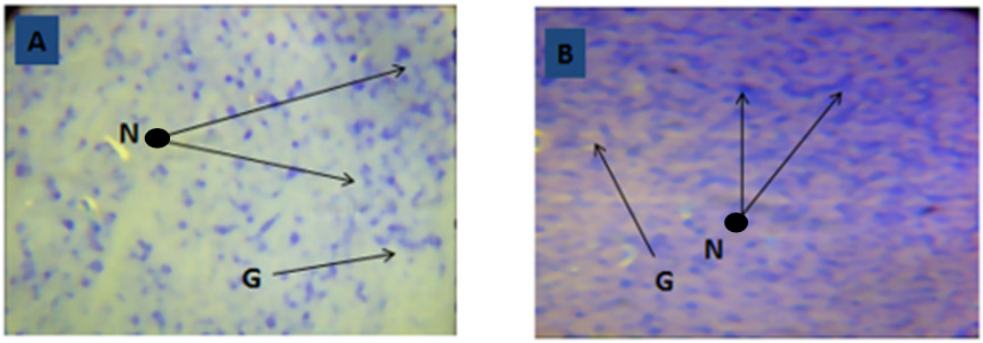

Fig. 3. Hypothalamic section of rat (A) and bat (B) with stained neuropil (N) and glial cell (G). CFV X100

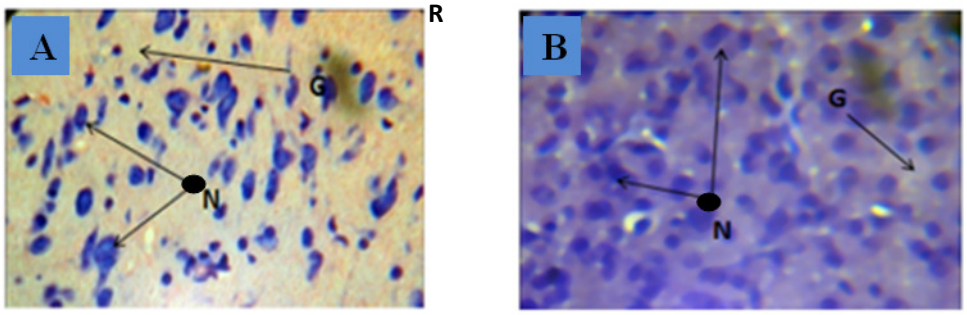

Fig. 4. Hypothalamic section of rat (A) and bat (B) with stained neuropil (N) and glial cell (G). CFV X400

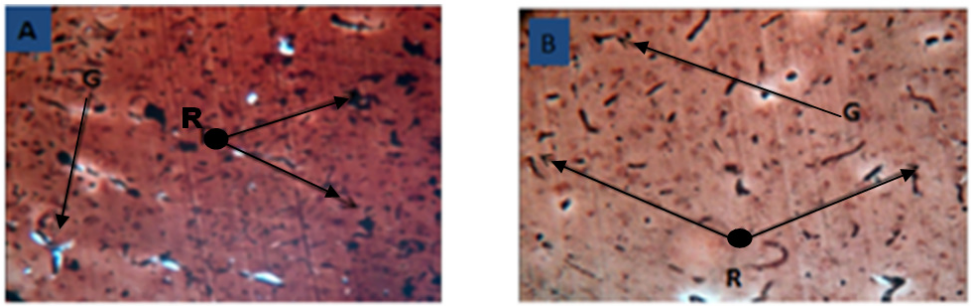

Fig. 5. Hypothalamic section of rat (A) and bat (B) with stained reticulin (R) & glial cell (G). G & S X100

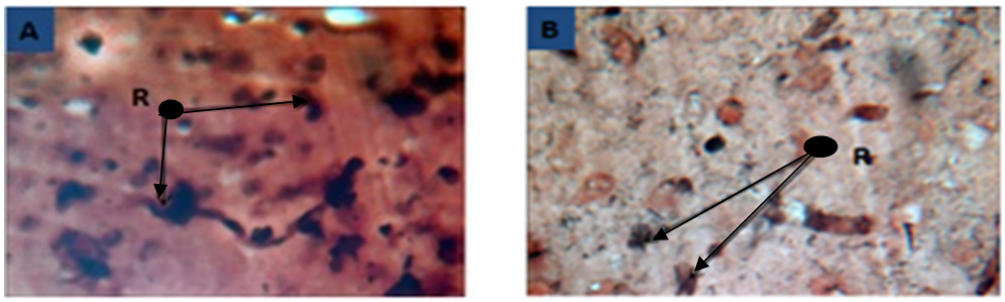

Fig. 6. Hypothalamic section of rat (A) and bat (B) with stained reticulin (R) & glial cell (G).G&S X400

Table 2. Shows the activities of enzymes in the hypothalamus in IU/L

Group	G-6-PD	LDH	ALP	ACP
Rat	1086±26.67	1135.00±0.41	829.75±1.31	558.00±0.41
Bat	429.75±3.15	615.50±0.65	1022.00±0.91	389.25±2.50

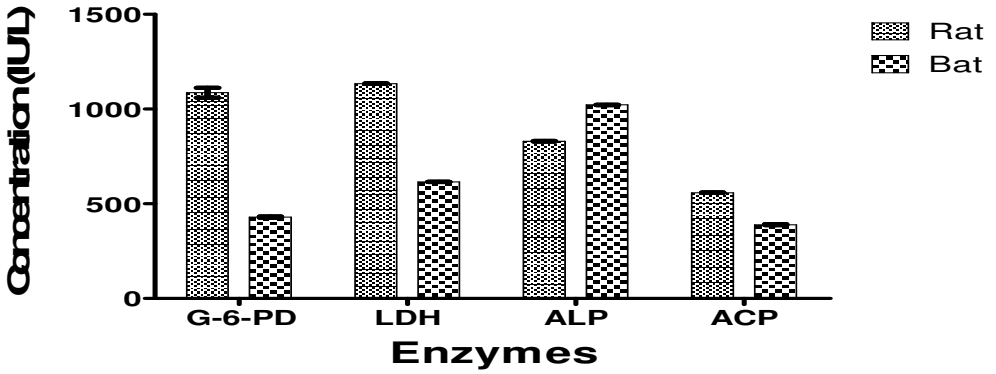

Chart 3. Comparing the activities of enzymes in the hypothalamus of both rat and bat

4. DISCUSSION

General observation on the macromorphological variation (body and brain weights) of the two mammalian species shows some differences, with bat having the higher mean of body weight 275.33±7.49, and its brain weight directly proportional to the body weight. Comparatively, it is observed from the Fig. 1 that, hypothalamus section of rat is deeply stained compared to bat, with more distinction and spatial orientation of the cellular layer of neuronal and glial cell in rat compared to discrete and almost uniformity in size, shape and distribution within the lamina layer of bat when observed with higher resolution of microscope. In the Fig. 3, there is a high population of neuron spreading within the mass of hypothalamus compared to that of bat indicated by the stained nissl substances at higher resolution, the distribution of nissl substances was observed to be particularly higher in rat, but moderate in bat. Though, the orientation of reticulin fibers in bat is regular compared to rat. Thus, the distribution and arrangement of the histological features (neuropil, glial cell and reticulinfibers) of hypothalamus found in the photomicrographs suggested that, the hypothalamus of rat undertakes more tasks than that of the bat [10]. This implies that, the hypothalamus of rat performs more functions of thermoregulation, feeding and circadian rhythm control than in bat [11,12]. However, the cells of the hypothalamus, that is, the lateral hypothalamic nuleus, arcuate nucleus and suprachiasmatic nucleus control feeding, satiety and circadian rhythm respectively. While rats are active during the day and night with higher mobility, they task their

hypothalamus more than that of the bat, the bats are active only at night. Thus, the high mobility of rats may demand for more homeostatic functioning of its hypothalamus. Nissl bodies are strongly basophilic inclusions found in the cell bodies of neurons. These granules are rough endoplasmic reticulum with free ribosomes and are the site of protein synthesis. They are thought to be involved in the synthesis of neurotransmitters such as acetylcholine [13], protein synthesis is also very crucial for the growth and development of every living cell and tissue of the body. Increased Nissl substances in the hypothalamus of rat and bat in the Fig. 4 suggested that, the cells are very active in protein synthesis. The distribution of reticulin suggested that, the fibers are well distributed in the cellular components of hypothalamus, therefore, the observation of the section of hypothalamus in Fig. 4 indicated that, the basement membrane of the hypothalamus is more developed than that of bat.

However, the measure of the activities of enzymes in the hypothalamus of the mammalian species further confirms the variations in the individual peculiarities and its diets. Thus, the rat which is omnivorous consumes higher dietary carbohydrate, and bat is frugivorous that consumes less dietary carbohydrate. The cells use glycolysis as the primary source of energy, since G6PDH can generate NAD+ rapidly, thereby promoting triose phosphate oxidation which is found to increase during the stress [14], increase in the activity of G6PDH indicates increased in carbohydrate metabolism for energy and ribose production via pentose phosphate pathway [15], there are differences in the values of the enzymes in the two mammals, the rat utilizes more energy for homeostatic, feeding and circadian rhythm control, followed by bat, this is supported by the histological observations in the photomicrographs.

In overexertion conditions when oxygen is absent or in short supply to cope with energy demands of the hypothalamus, LDH comes in to play the role of energy production. Increase in the activity of LDH indicates an increased in carbohydrate metabolism for energy production via glycolytic pathway and vice versa [16,17]. Based on the result obtained, the bats mostly combat with lower oxygen supply as they reside at high altitude. This is obvious as seen in their lifestyles; bats engage in true flight and employ an alternative pathway for metabolism [18].

The ALP activity in the hypothalamus was comparatively different between the mammals with the higher activity seen in rat and the lesser in bat. ALP mainly facilitates transport across cell membranes, causing the breakdown of ATP to ADP and inorganic phosphate, thereby making free energy available for metabolic process. This is important in animals that utilizes alternative pathway for energy production.

5. CONCLUSION

Considering the histological analysis and the enzyme activity, the hypothalamus of both animals are observed to be significantly different and being influenced by their feeding and lifestyles.

ETHICAL APPROVAL

All authors hereby declare that “Principle of laboratory animal care” (NH publication No. 85-23, revised 1985) was followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethic committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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