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# A comparative assessment of superior colliculus of Rat (*Rattus norvegicus*), Bat (*Eidolon helvum*) and Pangolin (*Manis tricuspis*): A histochemical study

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**ABSTRACT**

*In this study, the superior colliculus (SC) of rat, bat and pangolin were compared using histological and quantitative histochemical parameters to observe possible modification that enable these mammals to cope with their habitation particularly with respect to their diet. The study was conducted using ten adult Wistar rats, ten fruit bats and eight pangolins comprising of both sexes. After being sacrificed by cervical dislocation, their skulls were opened using bone forceps to expose the brains. The superior colliculi were excised from each brain, homogenized and read spectrophotometrically for the activities of lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), acid phosphatase (ACP) and alkaline phosphatase (ALP). The SC tissue sample meant for histological studies were fixed in 10% formol calcium and processed for paraffin wax embedding. Serial sections of 3µm thickness were stained with Hematoxylin and Eosin and Cresyl fast violet stains. The stained tissues were studied under the light microscope. Application of one-way ANOVA statistical analytical method showed that there were significant differences ($p<0.05$) in the activities of LDH, G-6-PDH, ACP and ALP of the SC of the three mammals as revealed in the quantitative histochemistry of these enzymes and markers. Histological observations revealed variations in the distribution of neurons and their supporting glial cells with the neurons in the SC of the rat appearing more numerous as well as in the pangolin and are fewer in the bat. The comparison of the differences observed in the histological and the quantitative histochemical activities in these mammalian species revealed a variation in the visual ability and their individual peculiarities in relation to their mode and pattern of living.*

**Keywords:** superior colliculus, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, acid phosphatase, alkaline phosphatase.

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## INTRODUCTION

The superior colliculus (SC) is the upper component of the *copora quadrigermina*. It is responsible for saccadic eye movement [1, 2] and relaying of visual impulses to and from the lateral geniculate bodies that will in turn terminate in the calcarine sulcus of the geniculo calcarine tract. It also plays a major role in balance via its interaction with the inferior colliculus in the copora and exchanged fibres between the medial and lateral geniculate body.

The SC also functions in visual attention and centering of the visual image on the retina, thereby serving essentially as a visual relay centre [3]. It transforms both visual and non-visual sensory signals into motor commands that control orienting behaviors [4].

The rat, bat and pangolin are nocturnal mammals [5]. The rat is omnivorous; bat is frugivorous while the pangolin is insectivorous.

G-6-PDH is the first and rate limiting enzyme in the pentose phosphate pathway. G6-PDH converts nicotinamide adenine dinucleotide phosphate ($NADP^+$) into its reduced form, NADPH, and glucose-6-phosphate is then converted into a pentose sugar (ribulose-5-phosphate). The 5-carbon sugar is a precursor of DNA, RNA, and ATP [6]. LDH catalyzes the conversion of pyruvate to lactate with concomitant oxidation of NADH during the last step in anaerobic glycolysis [7]. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply. G6PDH and LDH are enzymes of carbohydrate metabolism that are involved in aerobic and anaerobic pathway respectively for ATP production [8].

Acid phosphatase (ACP) is a family of enzymes that belong to the hydrolase class. It possesses the ability to catalyze the hydrolysis of orthophosphate monoesters under acidic conditions. ACP is the marker enzyme for lysosomes [9] since it is found particularly in lysosomes and secretory vesicles. It has been shown that lysosomes, acting presumably via their acid hydrolases, are involved in a variety of cytoplasmic degradative changes during physiological processes [10, 11] especially those involved in neurodegeneration. ACP has also been used to monitor cell death and cell lysis [12-15].

Alkaline phosphatase (ALP) is a group of catalytic proteins found in all body tissues sharing the capacity to hydrolyze phosphate esters in alkaline medium [16, 17]. ALP has been found to act opposite kinases, a function shared with endonucleases. ALP, a membrane biomarker and a regulator of DNA cleavage, mainly facilitates transport across cell membranes, causing the breakdown of ATP to ADP and inorganic phosphate, thereby making free energy available for metabolic processes [18].

The present work was therefore undertaken to compare the differences in the histological and quantitative histochemical activities in the SC of these mammalian species.

## MATERIALS AND METHODS

### Experimental animals

All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health [19]. Ten adult Wistar rats, ten fruit bats, and eight pangolins of both sexes were used for this comparative study. The adult Wistar rats were obtained from the animal holdings of the department of Anatomy of the University of Ilorin and sacrificed shortly after purchase. The bats were curled down from their roosting colony with the assistance of experts who possess State permit at the bats colony at the Flower Garden area of Government Reserve Area (GRA), Ilorin and were treated with tetracycline as prophylaxis against bacterial infection and sacrificed about five hours later. The pangolins were procured from Asejire, a village in the North West Area of Osun State and sacrificed before dark the same day. The G6-PDH LDH and ACP kits were purchased from Randox laboratory limited, UK and ALP from Quimica Clinica Aplicada's, Spain.

The animals were sacrificed by cervical dislocation. The skulls of the sacrificed animals were opened using bone forceps to expose the brain. The superior colliculi were located via tracing the optic tract to the optic chiasma and excised with the aid of the Atlas of the Rat Brain [20]. The specimens for routine histological investigations were fixed in 10% formol calcium and processed for paraffin wax embedding. Serial sections of 3µm thickness were stained with Hematoxylin and Eosin and Cresyl fast violet. Tissue sample meant for quantitative histochemical enzyme studies were preserved separately in cold 0.25M sucrose and were homogenized with Polter-Elvhjem homogenizer.

The homogenate were centrifuged at 5000rpm for 10 minutes. The supernatants were immediately stored in the freezer (-20$^o$C) and assayed within 48 hours. The activities of G-6-PDH, LDH, ACP and ALP were determined in the homogenate by the methods of Lohr and Waller (1974) [21], Wei (1975) [22] and Babson *et al.*, (1966) [23] respectively and were read spectrophotometrically.

### Statistical analysis

Values were reported as mean ± SEM. Significance was determined statistically by application of one-way analysis of variance (ANOVA) using statistical software SPSS version 17 at 95% confidence interval. Differences between means were considered statistically significant at $p<0.05$.

## RESULTS

It is observed in the sections stained in H & E as demonstrated in the Fig. 1, that the spatial orientation of the cellular layer differs. In the rat it was observed to be an instance of an alternating cellular and fibrous layer arranged in discrete fine units. In the bat, the orientation of the cells is towards the lamina layers almost interspersed in between the sheaths at most instances. The variation in cell types is less compared to what is seen in the rats and pangolin. The cell types are almost uniform in size, shape and distribution within the laminae. In the pangolin however, the distribution of cells is in the laminae of the superior colliculus, the cells seems to be more adapted for specific purposes as each loci of cell cluster was seen to have similar orientation and shape.

Using Cresyl fast violet (CFV) staining technique to compare the abundance of Nissl substance of the mammalian species as demonstrated in the photomicrographs labeled Fig. 2. The intensively stained Nissl substances are apparent; the nuclei in the cells are also visible.

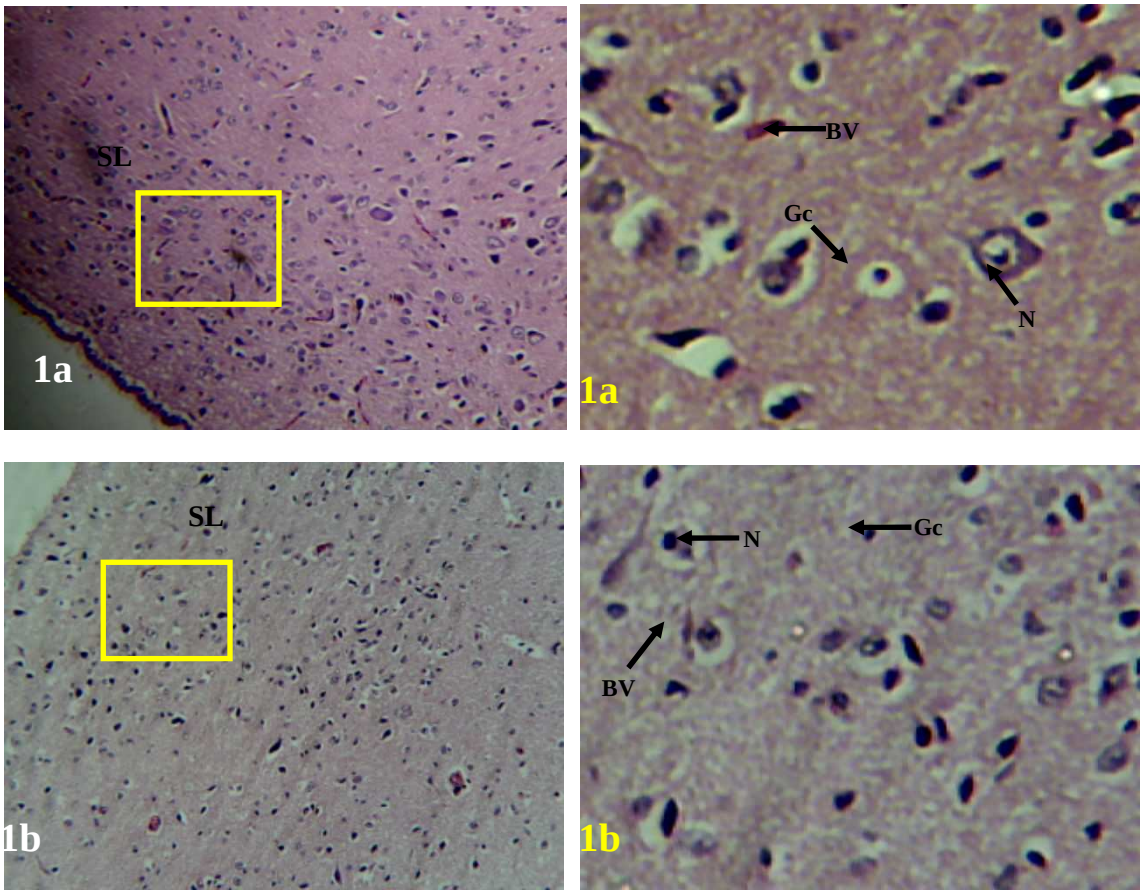
Result obtained from the present study show significant difference in the activities of the quantitative histochemistry (Tables 1).

**Table 1: Enzyme activities in the superior colliculus of the three mammals (IU/L)**

| Animals | G-6-PDH | LDH | ACP | ALP |
|---|---|---|---|---|
| Rat | 1191.66±4.40* | 1420.00±2.88* | 8.33±0.14* | 6.86±0.12 |
| Bat | 550.00±9.07* | 736.00±20.22* | 12.73±0.17* | 6.43±0.21 |
| Pangolin | 749.66±23.96* | 471.00±28.98* | 6.90±0.14* | 6.90±0.28 |

*(n=6) *level of significance (G-6-PDH p=0.000) (LDH p=0.000) (ACP=0.000) (ALP=0.318)*

## HISTOLOGICAL ILLUSTRATIONS



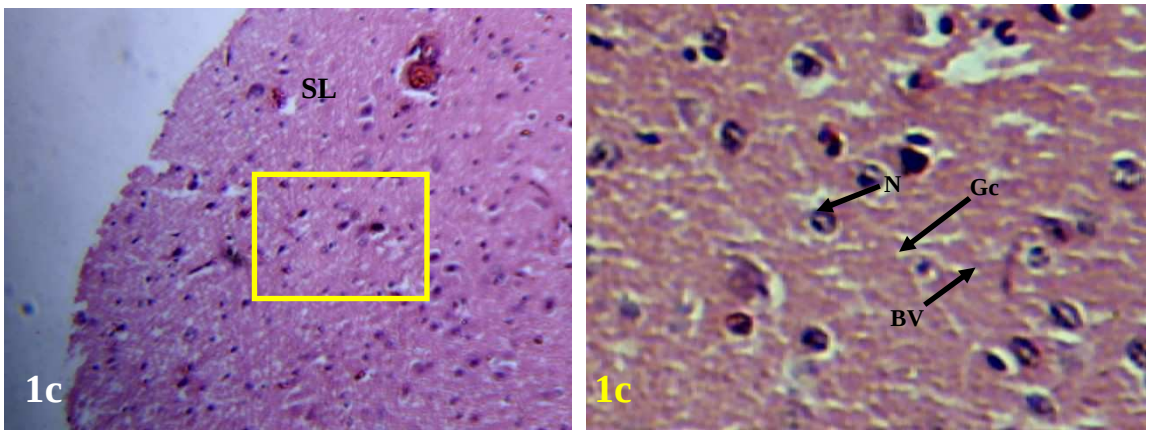
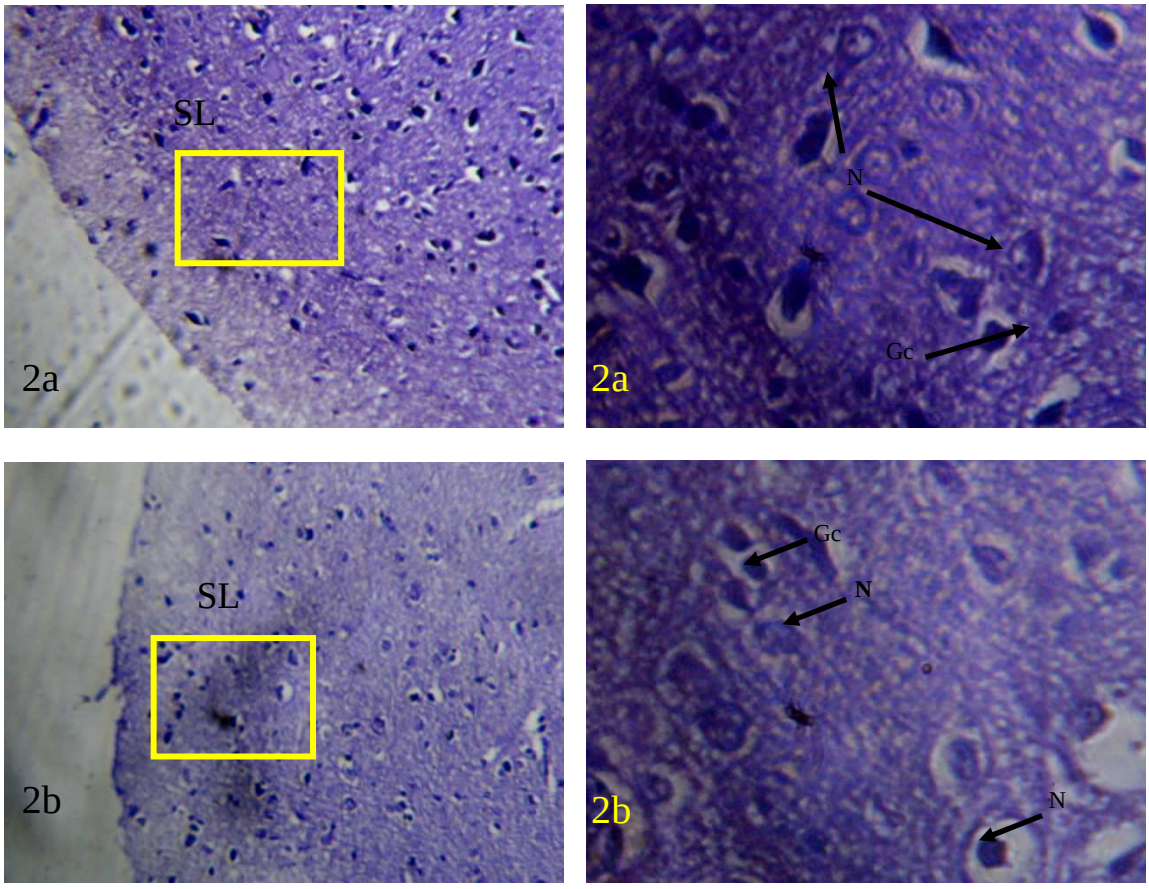



**Fig. 1: Photomicrographs of the superior colliculus of Rat (1a), Bat (1b) and Pangolin (1c).**
*Legends: SL=Superficial layer, BV=Blood Vessel, N=neuron, Gc=Glial cell. (H&E, x180 (left), x720 (right)). Note; the panoramic view of the yellow bordered region is presented on the right.*



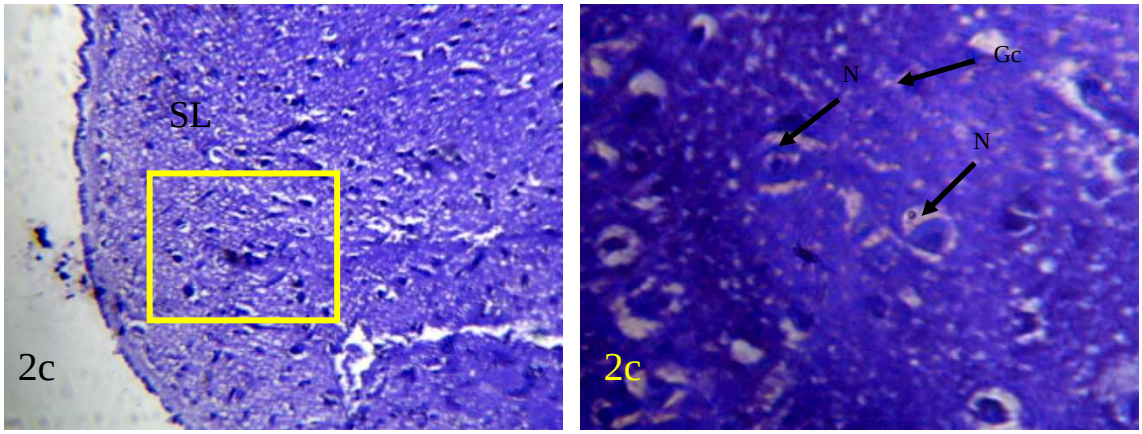



**Fig. 2: Photomicrographs of the superior colliculus of Rat (2a), Bat (2b) and Pangolin (2c).**
*Legends: SL=Superficial layer, N=neuron, Gc=Glial cell. (CFV, x180 (left), x720 (right)). Note; the panoramic view of the yellow bordered region is presented on the right.*

## DISCUSSION

Comparatively, the implication of the histological observations strongly suggests that the SC of rats and pangolins undertake more tasks than that of the bats. Suggesting that the rat SC perform the functions of visual attention and centering of image on the retina than in the bat and pangolin. Since the cells of the superior colliculus are involve in visual localization, movement and orientation of the eyes, accommodation and pupillary reflex [24]. The feeding habits, lifestyle as well as the nature of their habitat would account for this. While rats are active during the day and night with higher mobility, they task their SC more than that of the bat and pangolin. The pangolins are only active at night. However, being an insectivore, they require highly effective vision to identify and contrast food which could be quite small, although, olfactory communication also plays a significant role [25]. The fruit bats on the other hand require less rapid movement of their eyes in search for food. They feed on fruits and depend on the combined use of echolocation and olfactory clues to find ripe fruits hidden and nestled among leaves [26-31]. 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. 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 SC of the rat and pangolin, indicates that the cells are very active in protein synthesis while those of the bat are moderate.

The measure of the quantitative histochemistry in the superior colliculus of the mammalian species further confirms that the variations observed were due to their individual peculiarities. The cells use glycolysis as the primary source of energy [32]. Increase in the activity of G6PDH indicates increased in carbohydrate metabolism for energy and ribose production via pentose phosphate pathway. From the result obtained, the rat utilizes more energy for visual movement and orientation. This followed by pangolin and bat which support the histological findings. In over-exertion conditions when oxygen is absent or in short supply to cope with energy demands of the SC, LDH come in to play in energy production.

Increase in the activity of LDH indicates an increased in carbohydrate metabolism for energy production via glycolytic pathway. Based on the result obtained, the rat uses more energy to carry out functions of the SC. This is obvious as seen in their lifestyle. Bat engages in true flight and employs an alternative pathway for metabolism. This suggests why bat have a higher activity level compared to the pangolin. A closer look at the G6PDH:LDH in examining carbohydrate metabolism as a balance between aerobic and anaerobic metabolism shows;

The Rats G6PDH:LDH ratio reads 1191.66±4.40:1420.00±2.88 indicating the anaerobic system is more active than the aerobic system, while the same was observed in the Bats (550.00±9.07:736.00±20.22) with the levels of LDH being higher than those of G6PDH indicating a negative shift in oxygen usage for metabolism, while a positive shift was seen in the pangolin with the ratio reading positive 749.66±23.96: 477.00±28.98.

The ALP activity in the SC was comparable between the mammals with the highest activity seen in the pangolin, the rat then the least was observed in the 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 (Murray *et al.*, 2003).

The ACP activity in the SC was compared, the highest activity was seen in bat, the rat and pangolin had the least. The higher activity in bat may indicate higher amount of unused substances in form of precursors of neurotransmitters within the colliculi responsive to visual motion. It also help to "mop up" the phosphate ions produced as a result of ATP hydrolysis.

## CONCLUSION

The results of the measure of the histological observations and quantitative histochemistry revealed a relationship in the visual ability of the mammalian species in relation to their mode and pattern of living. This suggests that different mammals have evolved different mechanisms to cope with feeding and habitat differences.

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