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Comparative Neuroanatomical Characterization of the Cerebrum of Wistar Rat, Guinea Pig and Rabbit

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ABSTRACT

Introduction: comparative anatomy provides empirical facts for the classification of biological species on the basis of similarities or diversities of their anatomical characteristics. Animal models, especially rodents, are imperative tools for neuroscience research useful in elucidating brain pathologies. This study comparatively characterizes the anatomical features of the cerebrum of some rodent species; Wistar rat, *Cavia porcellus*, and Oryctolagus cuniculus using morphologic and microscopic assessments.

Material and Methods: nine adult rodents (n= 3/species): Wistar rat, guinea pig (*Cavia porcellus*), and rabbit (*Oryctolagus cuniculus*) were used. Morphological and microscopic assessments including morphometrics of brain dimensions, gyrification, encephalization quotient (EQ), histology, and histometry of cerebral M1 region were conducted and features compared amongst species.

Results: morphologically, brain dimensions revealed higher (p<0.05) values for rabbits among the rodent species. Gyrification was observed on the dorsal surface of cerebral hemispheres of the rodents, except in rats. Guinea pigs had the highest (p<0.05) EQ levels for species intelligence. Microscopically, layers III and V of cerebral M1 revealed similar population of cell types in all species. Histometric characteristics of pyramidal neurons in layer V were different (p<0.05) amongst the species with higher values in rabbits. No remarkable difference in cell distribution of cerebral M1 in layers III and V amongst the species.

Conclusion: there exists morphologic variation in the brain features of compared rodent species and a small-scale variation in the cytoarchitectural features of the M1 cerebrum of the species. Results obtained are beneficial in identifying suitable species as potential models for certain neuroscience research.

Keywords: Brain dimensions; Cell distribution; Encephalization quotient; Gyrification; Microscopy.

Introduction

Biologically, comparative sciences involve assessing the similarities and differences between two or more species and, anatomical sciences refers to the identification and description of bodily structures in relation to functions of an organism[1,2]. Comparative anatomical assessment of bodily structures or features aids the classification of organisms based on similar characteristics of their structures and provides for the classification of seemingly unrelated species as either phylogenetically related or otherwise[3,4]. Comparison of structures of the nervous system amongst two or more species at the macroscopic and/ or microscopic levels are the basis for comparative neurobiology/ neurosciences[5,6].

Animal models, especially small laboratory animals including rodents, are imperative tools for neuroscience research which are beneficial in elucidating neurological pathologies and development of possible therapies for neurological disease conditions where human subjects cannot be used[7-10]. The rodent species, rat, have made priceless contributions to biology and medicine; widely used in drug development and related therapies[11, 12]. Moreso, rats are the species of preference in biomedical research because of their significant degree of similarity with humans genetically[13,14]. A popular strain of laboratory rats, the Wistar rat, is an outbred albino rat established for over a century as a useful tool in biomedical researches[15-18]. The Wistar rat is currently one of the most popular animal models

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used for neuroscience research; a lot of effort has been made to describe the biology of the nervous system, especially the brain of the species[19].

A larger-than-rat rodent species, the guinea pig (*Cavia porcellus*), is a species belonging to the *Caviidae* family. This species has biological similarities to humans, thus beneficial in several fields of research[20-23]. Guinea pigs have been reportedly used as an experimental animal model in biomedical research including studies related to the immune and nervous systems[24-27]. Guinea pigs can learn complex paths to food and accurately remember a learned path for several weeks[28]. The species is not particularly agile amongst other characteristics that make it different from smaller rodents like rats and mice. However, this difference might present the guinea pig as a suitable tool for some neuroscience investigations, especially those related to movement and motor activity[7].

Larger than guinea pigs, the rodent species rabbits (*Oryctolagus cuniculus*) are small mammals of the family *Leporidae*. This species, like the rat and guinea pig, is commonly used as an experimental animal model in biomedical research, especially in the fields of immunology, pharmaceutics, and genetics[9,29]. Several unique features of the rabbit make it an excellent model for a number of human-related diseases[30-32]. The behavioral characteristic of rabbits including docility, non-aggressiveness, and ease of handling makes them a unique model form of certain neurological conditions. Additionally, rabbits are prey animals as such, constantly aware of their environments. Survival of predation is by burrowing and hopping away in a zig-zag motion and, delivering powerful hind limb kicks when captured[33,34].

Several studies have demonstrated the great contributions of these laboratory rodent species; rats, guinea pigs, and rabbits, in the advancement of medicine, and a number have described the biology, including the nervous system of these species[35-40]. Some studies have compared the morphologic features of the central nervous system, especially the brain and, a few, certain regions of the brain[41,42]. Established, the brain is anatomically diverse across species, demonstrating structural differences at both gross and microscopic levels over short phylogenetic distances[43-45]. Thus, there is a need to comparatively elucidate the anatomical features of the cerebrum of these rodent species in order to mark out possible similarities and differences, and identify suitable species as potential models for certain neuroscience research.

This study comparatively characterizes the anatomical features of the cerebrum of some mammalian rodent species; Wistar rat, *Cavia porcellus* (guinea pig), and *Oryctolagus cuniculus* (rabbit) using morphologic and microscopic assessments.

Materials and Methods

Experimental Animals

Nine adult male rodents, three of each species: Wistar rat (*Rattus spp.*), guinea pig (*Cavia porcellus*), and rabbit (*Oryctolagus cuniculus*), were obtained from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria and transferred using animal cages to the Animal House of Department of Human Anatomy, Faculty Basic Medical Sciences, College of Medical Sciences, ABU, Zaria. The rodents were acclimatized for 24 hours and humanly euthanized thereafter.

This study was conducted with consent from the Committee on Research Ethics, Department of Human Anatomy, Ahmadu Bello University, Zaria, and all experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Experimental Protocol

The rodents were grouped into three groups (A, B, and C; Wistar rat, Guinea pig, and Rabbit, respectively; n= 3) and weighed using a digital weighing scale (Electronic Scale SF-400, 0.1g). Rodents were euthanized using chloroform inhalation and the brains were carefully dissected out from the cranial cavity for subsequent studies (See Figure 1).

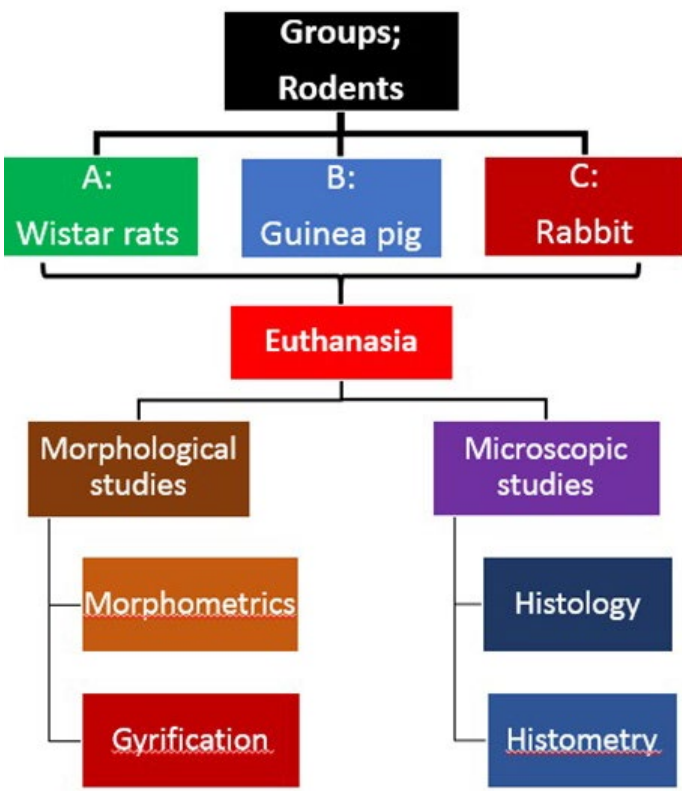

Figure1. Experimental protocol.

Morphological Studies

The harvested brains, in all the rodents, were observed (dorsal and ventral surfaces) for gross

features and different parts of the brain identified. Gross features including the number of grooves (sulcal depressions) on the cerebral dorsal surface and morphometrics involving measurement of brain dimensions were conducted and, outcomes were compared statistically among the studied rodent species. A brief description of the protocol adopted is as follows:

Gyrification: the number of sulci (grooves) on the dorsal surface of the cerebrum was determined by manual counting under bright lighting using a magnifying hand lens (Magnifying glass – 75 mm). Counting was done separately on the left and right hemispheres of the cerebrum.

Brain dimensions: dimensions of the cerebral region of the brain were determined. Dimensions measured were: length (antero-posterior most prominent points), width (right-left most prominent points), and thickness (dorso-ventral width; most prominent points). Measurements were conducted using an electronic digital caliper (Electronic Digital Caliper Vernier – 150 mm LCD). (See Figure 2).

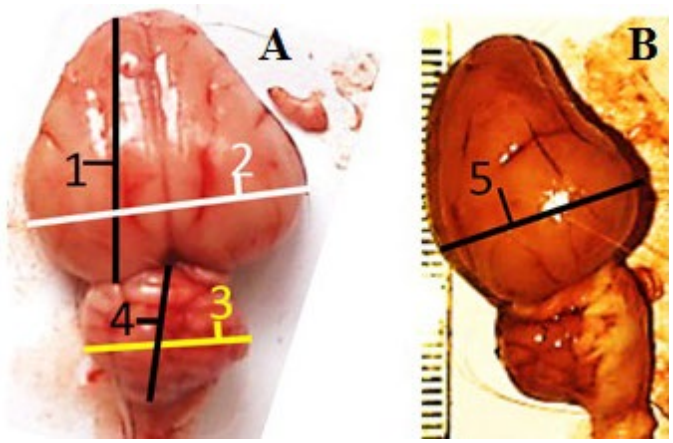

Figure 2. Measurement of brain dimensions.
Dorsal view (A) and lateral view (B); cerebral length (1); cerebral width (2); cerebellar length (3); cerebellar width (4); dorso-ventral cerebellar width.

Brain weight and organosomatic index: the harvested whole brains were weighed using a digital weighing scale (Acculab VICON; VIC-303, USA, 0.001 g) and organosomatic index computed (brain weight/ absolute body weightx100[40,46,47].

Encephalization quotient (EQ): level of intelligence of each species was determined by computing for EQ as described by Jeison[48] using brain and body mass parameters for mammalian species[49]

Microscopic Studies

Harvested brains were fixed in a fixative, Bouin's fluid and processed for microscopic assessments using histological and histometric techniques. A brief description of the protocol adopted is thus:

Histological Assessment

Fixed brains were processed using histological techniques, stained with Hematoxylin and Eosin (H & E) stains for light microscopic examination of the cerebral motor cortex (M1 and M2 regions). To obtain tissue sections of the cerebral M1 and M2 regions, guided by Rat Brain Atlas[19], the cerebrum was sectioned coronally at a point 7.4 mm posterior to the most anterior point of the cerebrum in Wistar rat. (See Figure 3). The corresponding points in relation to cerebral length in the other rodent species were computed and coronal sections made to target the cerebral regions of interest.

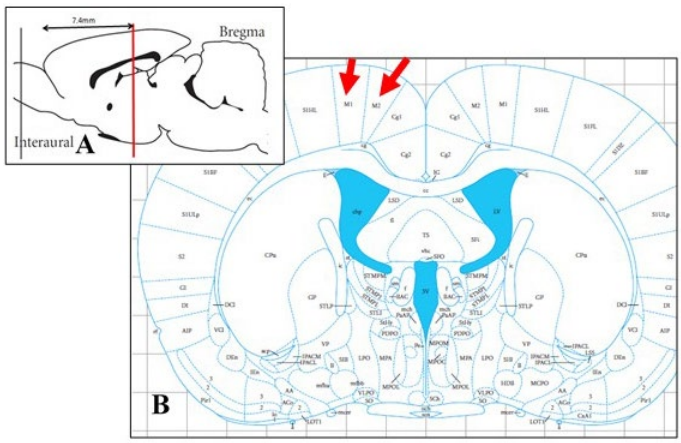

Figure 3. Sectioning and identification of brain region.
Point of coronal section (red line) (A) and red arrows indicating the M1 and M2 regions of the cerebral cortex (B). Primaary motor area (M1); secondary motor area (M2). Adopted from George Paxinos and Charles Watson Rat Atlas 6th edition 2007.

Histological (cytoarchitectural) features of the cerebral cortex, at different microscopic magnifications, were compared among the rodent species. Histological tissue processing was carried out in the Histology Unit of the Department of Human Anatomy, ABU, Zaria. Light microscopy and micrography (*using a digital microscopic camera*, MA 500 *AmScope*®, USA) was carried out in the Microscopy and Stereology Research Laboratory of the same facility.

Histometric and Cell Distribution Analysis

Histometry was conducted according the method described by[50] as an objective base for quantitative comparison of two dimensional (2D)-cytoarchitectural observation[51,52]. This involved measuring the soma (perikaryon) area and perimeter of pyramidal cells (neurons) of layers III and V of the cerebral M1 region using a light microscope (HM-LUX, LeitzWetzlar, Germany) with a 25/ 0.5 × objective (× 250 magnification) and, a micrometer slide (1 mm graduated in 0.01 mm units; that is divided into 100 µm units) and a computer running imaging software (AmScope MT version 3.0.0.5, USA) according to the manufacturer's instruction.

Cell distribution (density) in cerebral M1 (layers III and V) region was measured from micrographs (digital microscopic images; captured at × 250 magnification) using a computer running image analysis software

(ImageJ, NIH, US). The ImageJ Threshold Tool (threshold color: Black; color space: HSB) was employed according to the manufacturer's instruction and, the mean values for measured selected areas were computed and analyzed (See inset Figure 10). Data obtained were statistically compared among the rodent species studied.

Data Analysis

Data obtained were expressed as mean ± S.E.M and presence of significant differences among means of the groups were determined using one way ANOVA with Tukey post hoc test for significance. Values were considered significant when $p< 0.05$. Data analysis was conducted using the statistical software, Statistical Package for the Social Sciences (IBM SPSS *v* 21.0 SPSS Inc., Chicago, USA) and Microsoft Office Excel 2013 for charts.

Results

Morphological Assessments

Assessment of absolute body weight revealed significantly ($p<0.05$) higher value for rabbits compared to the other rodent species (Figure 4).

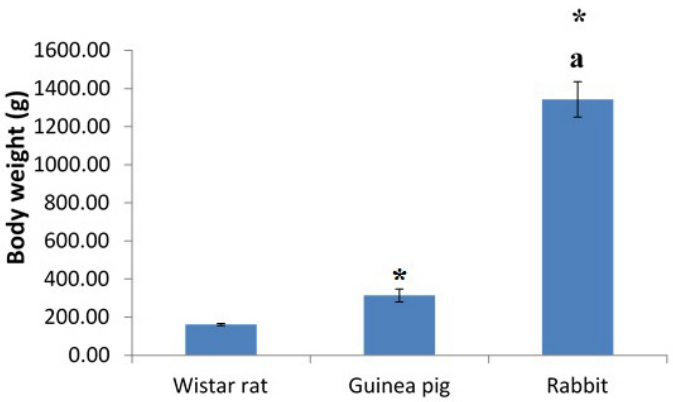

Figure 4. Comparison of absolute body weight of rodents.
n=3; mean± SEM; one way ANOVA tukey post test; *=$p<0.001$ and a=$p<0.01$ significantly different when compared to Wistar rat and Guinea pig, respectively.

Brains of the rodents were observed to be milky in color and presented with two major depressions on the dorsal surface; one coronal plane-oriented, separating the cerebrum (fore brain) from the cerebellum (hind brain) and, the other a sagittal plane-oriented, separating the cerebrum into two hemispherical halves. The ventral surface presented with distinct parts of the brain including the brain stem structures delineated by depressions and other accompanying features (Figure 5).

Gyrification

Sulcal depressions (grooves) were observed on the dorsal surface of cerebral hemispheres of the rodents, except in Wistar rats (Figure 6). Sulci were more frequent in rabbits compared to guinea pigs (Table 1).

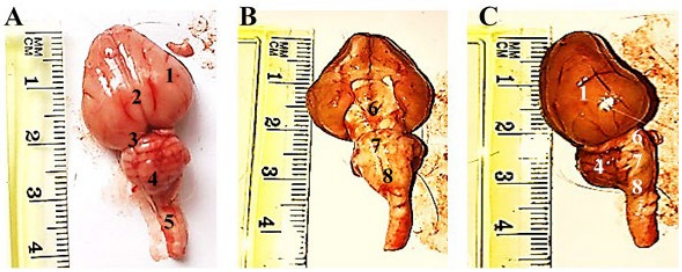

Figure 5. Guinea pig brain.
Dorsao view (A); ventral view (B); lateral view (C); cerebrum (1); intercerebral groove, separating the cerebral hemispheres (2); depression (groove) separating cerebrum from cerebellum (3); cerebellum (4); spinal cord (5); midbrain area (mesencephalic region) (6); pons (7); medulla (8).

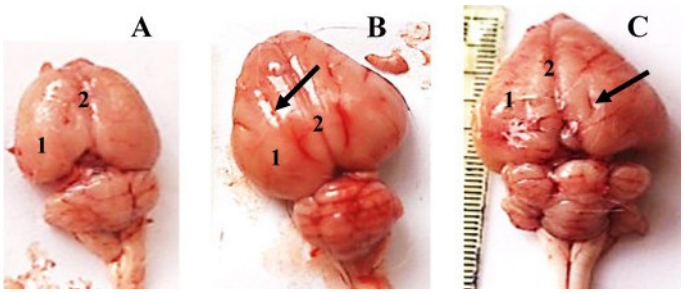

Figure 6. Sulcal depressions (grooves) on the dorsal surface of cerebrum.
Wistar rat (A); guinea pig (B); Rabbit (C); cerebrum (1); intercerebral groove (2); arrow indicating dorsol surface cerebral groove (sulcal depressin).

Table1. Cerebral sulcal depression of rodents

Cerebral hemisphere	Wistar rat	Guinea pig	Rabbit
Left	0	4.00±0.00	7.00±0.00
Right	0	3.67±0.33	7.00±0.00

n=3, mean ± SEM

Brain Dimensions

Assessment of cerebral dimensions revealed remarkable ($p<0.05$) difference between species. Rabbit had the highest values for all the parameters measured (Figures 7a - 7c).

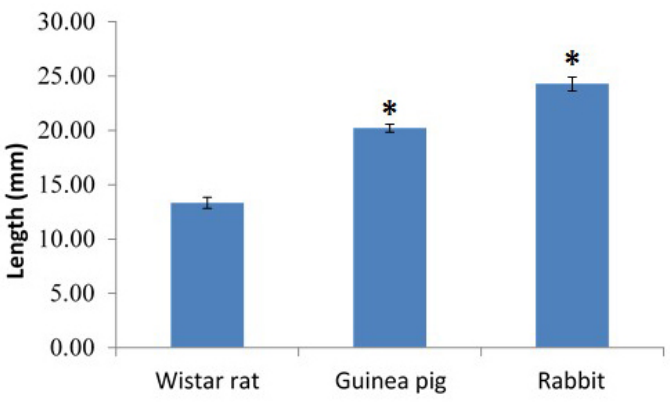

Figure 7a. Comparision of brain dimension (cerebral length) of rodents.
n=3; mean ± SEM; one way ANOVA Tukey post test; *=$p<0.001$ significantly different when compared to Wistar rat.

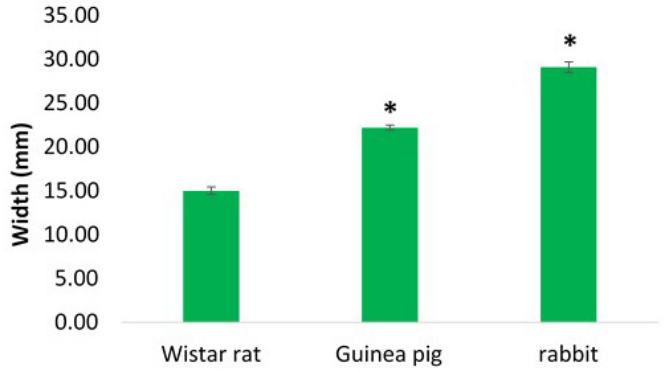

Figure 7b. Comparision of brain dimension (cerebral width) of rodents.
n=3; mean ± SEM; one way ANOVA Tukey post test; *=p<0.001 significantly different when compared to Wistar rat.

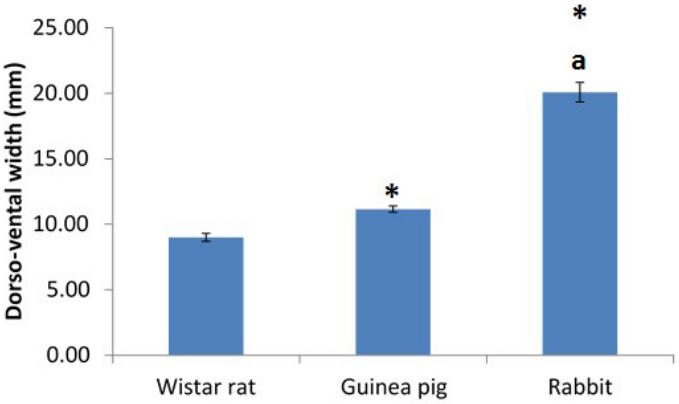

Figure 7c. Comparision of brain dimension (cerebral dorso-ventral width) of rodents.
n=3; mean ± SEM; one way ANOVA Tukey post test; *=p<0.001 significantly different when compared to Wistar rat and Guinea pig, respectively.

Brain weight and organosomatic index

Whole brain weight assessment revealed significant difference between species, with rabbit having the weightiest brain (Figure 8a). Comparison of organosomatic index between the rodents revealed remarkably (p<0.05) lower value for rabbit relative to Wistar rats. Guinea pig had the highest index value but was not significant when compared to Wistar rat (Figure 8b).

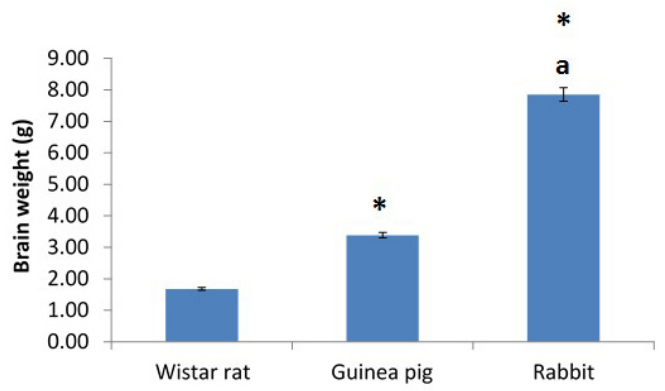

Figure 8a. Comparision of whole brain weight of rodents.
n=3; mean ± SEM; one way ANOVA Tukey hoc test; *=p<0.001 and a=p<0.01 significantly differnte when compared to Wistar rat and Guinea pig, respectively.

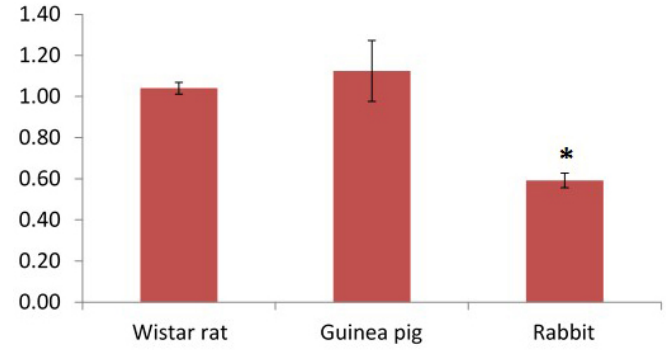

Figure 8b. Comparision of organosomatic index of rodents.
n=3; mean ± SEM; one way ANOVA Tukey hoc test; *=p<0.05 significantly different when compared to Wistar rat.

Encephalization Quotient

Assessment of EQ levels for species intelligence revealed remarkable (p<0.05) differences among species, with guinea pigs having the highest values (Figure 9).

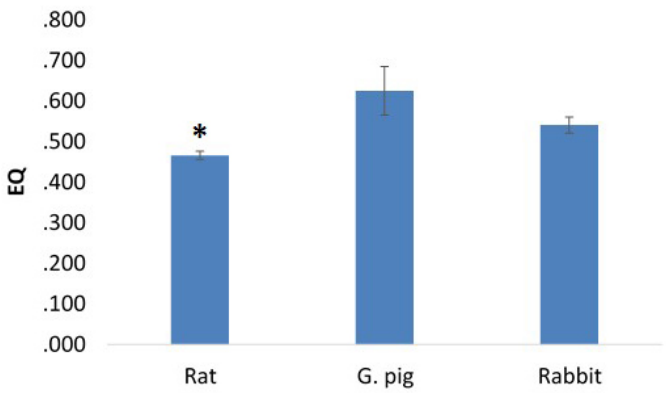

Figure 9. Comparision of encephalization of rodents.
n=3; mean ± SEM; one way ANOVA Tukey post test; *=p<0.05 significantly different when compared to Guinea pig. Encephalization Quotient (EQ).

Histological Assessments

Histological examination of cerebral sections of the rodent species revealed cortical cerebral regions, M1 and M2, laterally related to the median depression separating the two halves of the cerebrum at a low (microscopic) magnifying power. At higher magnifying power, cytoarchitectural features were observed and compared between species (Figure 10).

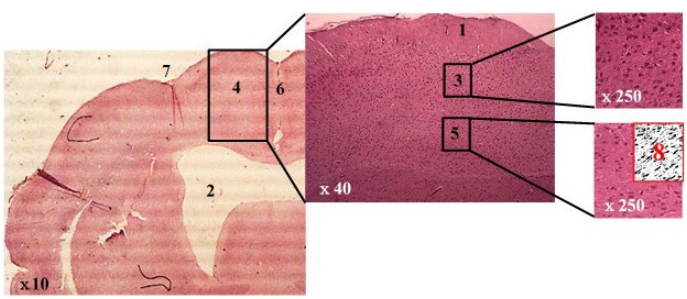

Figure 10. Coronal section of the cerebrum of Guinea pig with cortical regions. H&E stain.
Molecular layer of cerebral cortexn(1); lateral vetrilicle(2); outer pyramidal layer (5); median depression (groove), separating the halves of the cerebrum (6); sulcal depressions (7) inset, image analysis for cell distribution (8).

The cytoarchitecture of cortical cerebral regions presented with a variety of cells ranging from neurons to neuroglia, organized into cellular layers (laminae) in all the species. Six distinguishable layers (I - VI) were observed: the first, molecular layer (I) consists of few cells; a dense population of stellate and other cells make up the second layer called the external granular layer (II); the third layer, external pyramidal (III) consists of pyramidal cells; the fourth layer, internal granular layer (IV), consists of many stellate cells; the internal pyramidal layer (V), the fifth layer consists of many pyramidal cells, while the sixth layer, the polymorphic layer (VI) consists of numerous cell types including pyramidal and stellate cells. Ventral to the sixth layer, a thin layer of white mater was observed to delineate cerebral cortical from subcortical regions (Figure 11).

Assessment of layers III and V of cerebral M1 region revealed similar population of cell types, especially pyramidal neurons, in all species. However, difference in cell distribution (cellularity) was observed among the species, with the rabbit having a somewhat lesser distribution of cells relative to the other two species (Figures 12 and 13).

Histometric Analysis

Histometric characteristics (soma area and perimeter) of pyramidal neurons of cerebral M1 region (layers III and V) revealed the following:

The mean soma area and perimeter of pyramidal neurons in layer III showed no remarkable difference among the species (Figures 14a and 14b). The soma area and perimeter of pyramidal neurons in layer V were

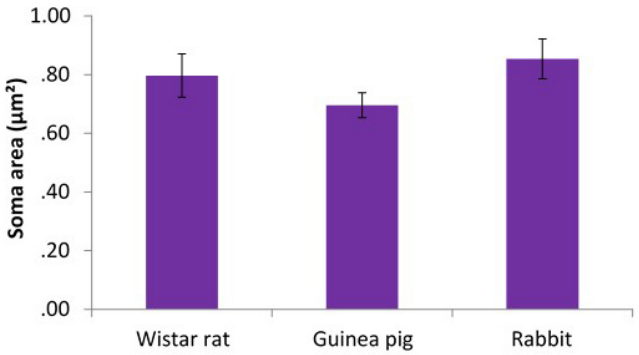

Figure 14a. Histometric characteristics (soma area) of pyramidal neuron in layer III (cerebral M1 region).
n= 20 ± 5 neurons, mean ± SEM; one way ANOVA Tukey post hoc test; no significant difference when values were compared between species.

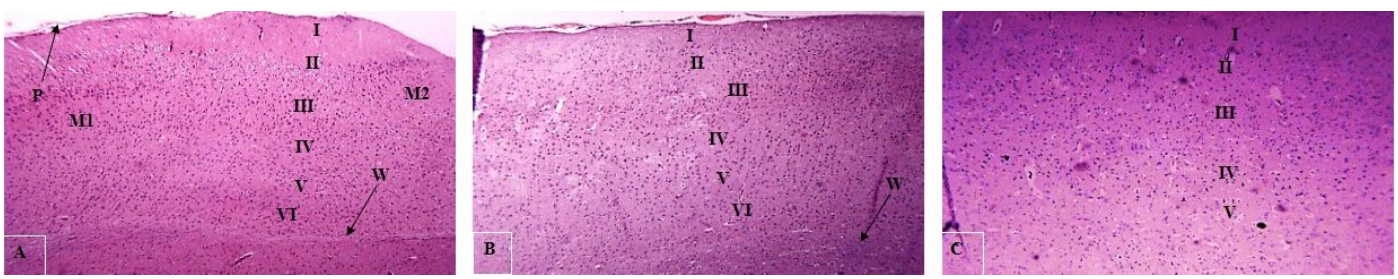

Figure 11. Coronal section or cerebral motor cortex of (A) Wistar rat (B)Guinea pig (C) Rabbit. H&E stain. Mag x40.
Primary motor area(M1) ; secondary motor area (M2) ; Pia mater (P); white mater (W); I: molecular layer; II: outer granular layer; III: outer pyramidal layer; IV: inner granular layer; V: inner pyramudal layer; VI: polymorphic layer.

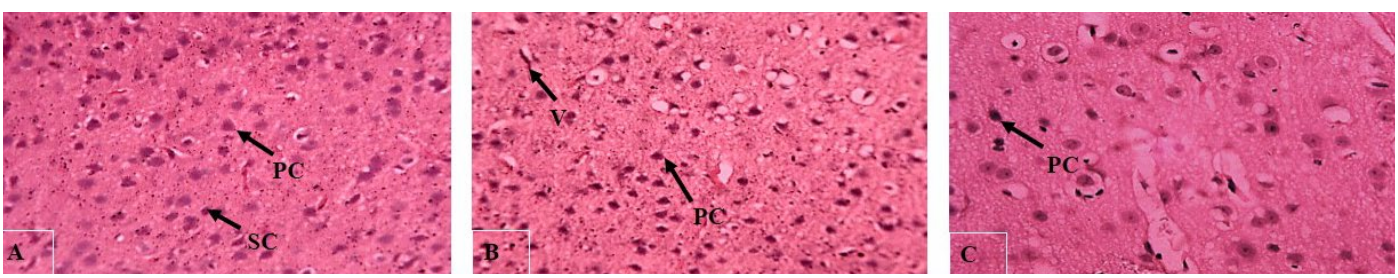

Figure 12. Coronal section or cerebral cortex (layer III, M1 region) of (A) Wistar rat (B)Guinea pig (C) Rabbit. H&E stain. Mag x250.
Primary cells (PC); stellate cells (SC); vessel (V).

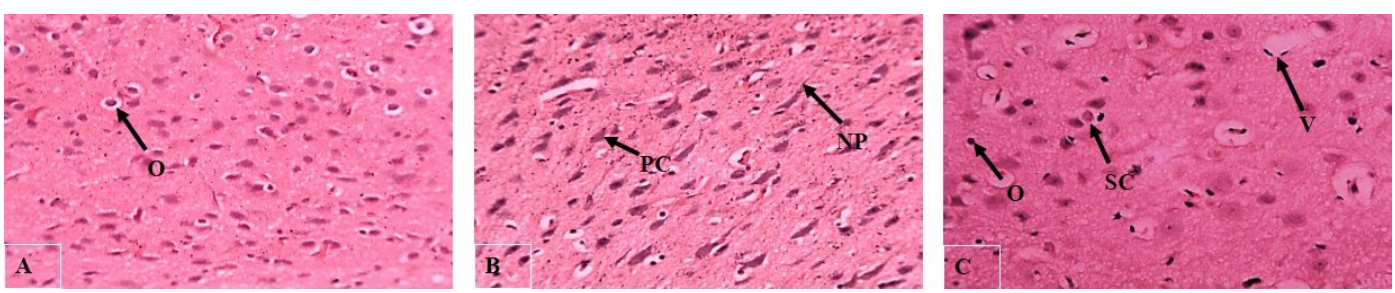

Figure 13. Coronal section or cerebral cortex (layer V, M1 region) of (A) Wistar rat (B)Guinea pig (C) Rabbit. H&E stain. Mag x250.
Neuronal process (NP); oligodendrocyte (O); pyramidal cells (PC); stellate cells (SC); vessel (V).

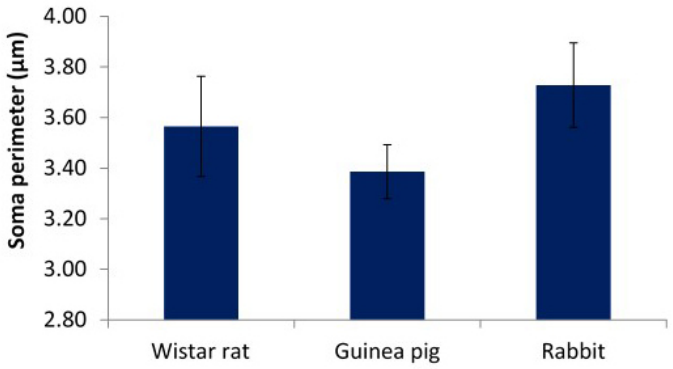

Figure 14b. Histometric characteristics (soma perimeter) of pyramidal neuron in layer III (cerebral M1 region).
n= 20 ± 5 neurons, mean ± SEM; one way ANOVA Tukey post hoc test; no significant difference when values were compared between species.

observed to be significantly (p<0.05) different amongst the species with higher values in rabbits (Figures 15a and 15b).

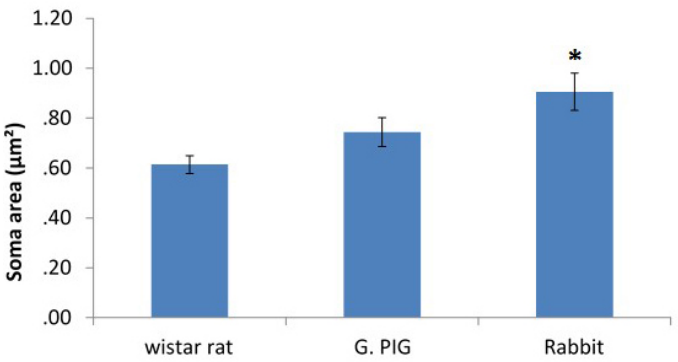

Figure 15a. Histometric characteristics (soma area) of pyramidal neuron in layer V (cerebral M1 region).
n= 20 ± 5 neurons, mean ± SEM; one way ANOVA Tukey post hoc test; *=p<0.05 when compared whith Wistar rat.

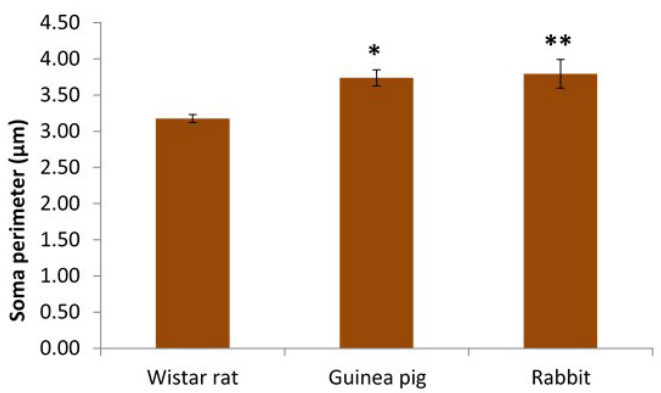

Figure 15b. Histometric characteristics (soma perimeter) of pyramidal neuron in layer V (cerebral M1 regionl).
n= 20 ± 5 neurons, mean ± SEM; one way ANOVA Tukey post hoc test; *=p<0.05, **=p<0.01 when compared whith Wistar rat.

Cell Distribution Analysis

Quantification of cell distribution in the cerebral M1 region revealed no remarkable difference in layers III and V amongst the species (Figures 16a and 16b).

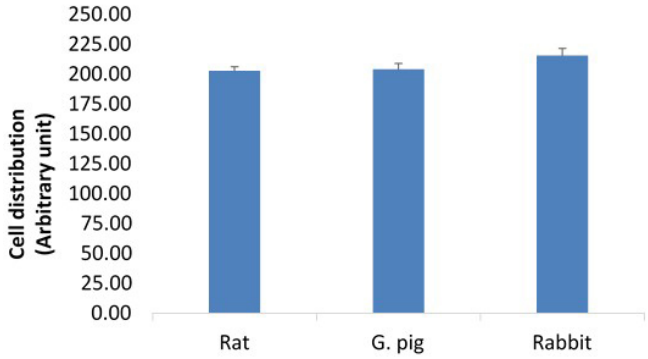

Figure 16a. Cell distribution in layer III of cerebral M1 region.
Mean ± SEM; one way ANOVA Tukey post hoc test; no significant difference when values were compared between species.

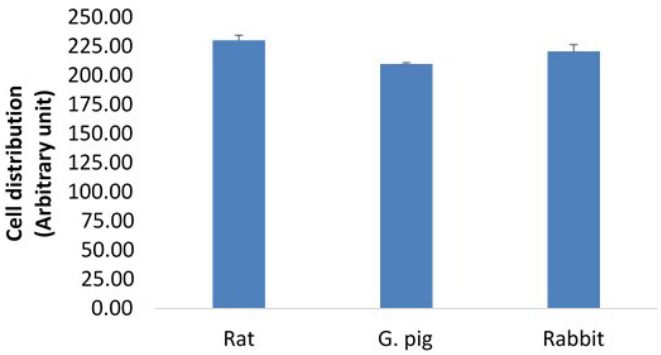

Figure 16b. Cell distribution in layer V of cerebral M1 region.
Mean ± SEM; one way ANOVA Tukey post hoc test; no significant difference when values were compared between species.

Discussion

In this study, the anatomical features of the cerebrum among three rodent species (Wistar rat, guinea pig, and rabbit) were examined and compared using several approaches including morphologic and microscopic assessments.

Morphological Studies

The absolute body weight for rabbits observed to be weightiest among the rodent species is in line with the reported trend that, larger body size tends to larger body mass[41,53]. Comparative studies have reported the mean weight values for smaller rodents including murines to be lower than mean absolute body weight values for larger rodents like *Cricetomys gambianus* (African giant rat); > 1 kg[54], *Thryonomys swinderianus* (grasscutter); > 2 kg[55] and porcupine; > 7 kg[56]. Musa *et al.*[41] reported an adult rabbit's body weight to be between 1 - 2 kg, which was the case in this study.

Milky appearance as coloration for brains of the rodent species is in line with reported brain coloration for rodents[41,53]. Milky to whitish coloration is a common feature for structures of the central nervous associated with the presence of high lipid components[57,58]. Fissural depressions separating the forebrain and hindbrain and, hemispherical halves of the forebrain observed in the rodent species are in agreement with the reported features for rodents' brain[41,59,60]. A ventrally

located brain stem observed in the rodent species is in agreement with reported morphologic features in rodents and other mammalian species[54,61]. During mammalian brain development, the mesencephalic and rhombemcephalic structures are commonly ventrally situated to procencephalic structures, especially the telencephalic structure; forebrain[38,39,42,62].

The cerebral cortex in mammals presents with variations across species, ranging from a small and smooth cortex (lissencephalic) commonly reported in rodents[59,62,63] and small primates (such as marmosets)[64] to moderately and profoundly convoluted cortex (gyrencephalic) reported in large rodents (such as capybaras)[64,65] and most primates, cetaceans, and ungulates[66,67]. In this study, sulcal depressions were observed on the cerebral cortex of guinea pigs and, more frequently, rabbits. Findings are in line with reported gyrencephalic cortex in smaller rodents including the agoutis and guinea pigs presenting with fewer and simpler patterns of gyri on cerebral cortical surface[62,63]. Ibegbu *et al.*[53] reported more frequent sulci in rabbits compared to other rodent species like African giant rats. Conversely, findings are at variance with the generally reported smooth cortical surface for these species, classifying them as lissencephalic; Müllhaupt *et al.*[37] categorized the rabbit brain as lissencephalic brain type in contrast to the gyrencephalic (convoluted) brain type. Musa *et al.*[41] reported the cerebral hemispheres of guinea pigs and rabbits to lack prominent gyri and sulci and placed the species in the lissencephalic group. Pardo *et al.*[60] observed several fissures, including lateral sagittal fissure, on the cerebral hemispheres of the rabbit brain, but reported a lack of gyri and sulci relative to prominent features in gyrencephalic species like dogs and nonhuman primates. These cerebral fissures were described as gyrification by Müllhaupt *et al.*[37] and related fissural frequency to both brain and body sizes of the animal. Variance observed in this study could be attributed to adaptive variation in species probably influenced by genetic and/ or environmental factors[62]. Extreme diversification of the cortical cerebral region of the brain in both size and morphology, including distinct patterns of convoluted ridges and grooves, have been reported among mammals[67,68]. Thus, guinea pigs and rabbits could be classified as simple gyrencephalic mammals having cerebral sulci corresponding to the primary sulci of primate[69,70]. Convolution of the cerebral cortex allows for a larger cortical surface area which has been associated with greater cognitive functionality and higher intelligence[62,71].

Rabbit manifesting with highest values for all the measured cerebral dimensions compared to the other rodent species corroborates the observed weightiest brain mass for the species; remarkably higher relative to the compared species. This finding is in agreement with the reports that associated larger brain dimensions and sizes with brain weights in different species[41,53]. In mammals, brain size tends to increase with the increasing size of the cranial cavity. Olude *et al.*[72] and Ibe *et al.*[59] reported a significant difference in brain dimensions for different brain weights of rodent species across age groups. Thus, larger body sizes tend to larger cranial cavities which in turn tends to larger brain sizes.

The observed weightiest brain mass for rabbits amongst the compared rodent species is in line with the trend reported for mammals; larger organ weights are associated with larger body sizes[73-76]. The mean brain weight value for an adult rabbit in this study is greater than that reported for murine[77,78], hamsters, squirrel[78,79], guinea pigs[41] and African giant rat[55,72], nonetheless lower than mean brain weight values reported for African grasscutter[55,59] and porcupine[80], which have larger body masses. Seyfarth and Cheney[44] reported a common scaling principle that brain size increases with increasing body size across the animal kingdom. Moreover, brain size has been associated with factors like complex habitats, specialized diets, and nocturnal behavior in rodents[55,73]. Thus, the difference in brain weight observed amongst the rodent species is suggestive of ecological, physiological, and behavioral differences among the species.

The organosomatic index, in this case, the brain-body weight ratio is a metric that quantifies the percentage of brain mass relative to the absolute body weight of a species[59]. Established, brain-body weight ratios differ from one taxon to another[44,48]. In this study, the guinea pig revealed higher values for brain-body weight ratio compared to the other species. The finding is in line with reported higher values for brain-body weight ratio in smaller rodents including mice and rats[50,72,81] in comparison to lower values reported for larger species like African giant rat[59,61,72] and African grasscutter[59]. Higher values for brain-body weight ratio have been associated with intelligence in mammalian species as larger relative brain weight provides for more complex cognitive tasks, including behavioral flexibility, social interactions, and survival advantage in novel environments[74,81-83]. Thus, findings are suggestive of Wistar rats and guinea pigs as more intelligent species and could be beneficial as animal models for cognitive-related investigations. Additionally, findings corroborate the established benefit of these species as suitable animal models for neuroscience researches[62,84].

Encephalization level is a relative brain size measure that serves as a more refined metric for comparing intelligence in relation to complex behaviors of different species in contrast to the direct values for brain-body mass ratios[49,76,85]. The highest value for encephalization observed in guinea pigs corroborates the findings of the organosomatic index and intelligence of the species compared to the other rodent species. The finding is in line with encephalization levels reported for guinea pigs[82,86] which is higher compared to some mammalian

species including rats, rabbits[55], African giant rats, and grasscutter[55,59], but lower compared to others including cats, dogs, and primates[48,49,86-88]. Additionally, the finding is suggestive and/ or corroborates the relevance of guinea pigs as a neurobiological research tool for certain investigations. Some researchers have demonstrated the potential of guinea pigs to provide a robust model with respect to multidimensional brain-behavior interactions that are relevant to human behavior[7,89] Lee *et al.*[7] reported that guinea pigs may be more suitable for research that relates to brain processing, sleep and fear-conditioning as data from this species are more easily translated to humans.

Although brain size is a traditional metric for intelligence, findings from this study agree with the established trend that brain weight, rather than brain dimensions, is a significant index of intelligence in mammals (Reiling, 1999; Steinhausen *et al*., 2016; Saganuwan, 2021).

Microscopic Studies

In this study, similar histoarchitectural features of the cortical cerebral region observed across the rodent species imply a phylogenetic relationship; having similar ancestry or taxon (class), mammalia[12,33,42]. A variety of cell types including glia and neuronal cells, especially pyramidal cells are characteristic to motor cerebral cortex[38,50]. Pyramidal cells in the cerebral M1 region are critically involved in the circuitry of motor-related functionality in mammalian species[39,90,91]. Findings are in line with the general histological features of rodents' cerebral cortex having cortical laminae as reported in murine, guinea pigs, African giant rat, and grasscutter amongst others[41,59,60].

Histometric quantification of 2D-histological data is an important tool that provides an objective basis for comparison of histological observation[50,52]. Remarkable differences in the histometric characteristics of pyramidal neurons of the M1 cerebrum, especially at layer V is suggestive of variation in neuronal sizes amongst the compared species which could be a reflection of motor functionality including the ability of rabbit to stand on its hind limb during environmental exploration and staying alert for predators[92,93]. This corroborates the finding of more frequent sulcal depression in this species. Sun and Hevner[65] reported that in mammalian species, the extent of cortical cerebral folding is a critical factor that influences sensorimotor skills and other abilities.

Cells vary greatly in size relative to cellular functionality rather than the size of the organism[94]. Findings is in line with studies that reported some cells including neurons can be longer and larger in larger animals compared to that of smaller animals[95,96]. Conversely, findings are at variance with reports that associated numerous cell numbers rather than cell sizes with larger bodily organs in animals[97,98]. Additionally, variation in neuronal sizes could be a result of the method of data collection; dimensions of 2D cell profiles were analyzed. 3D analysis of cell profiles such as stereology may provide different data[99,100].

Cell distribution is critical in the homeostasis of a tissue or organ as this reflects functionality in a biological system[101,102]. Variation in cell distribution has been reported in mammals in association with different body masses[98,102,103]. In this study, the absence of remarkable difference in cell distribution of the referred layers (layers III and V) of the M1 cerebrum amongst the species is suggestive of a convergent phylogenetic relationship. Histometric quantification of cell distribution objectively provides for precision compared with a direct visual appraisal of certain histological changes and improves assessment[50,51]. Thus, findings clarify and contradict the assertion of difference in cell distribution observed from a direct visual appraisal of histological sections. On the other hand, findings corroborate the idea of differences in neuronal sizes rather than a difference in cell distribution with respect to different body masses. A slight difference in sizes of analogous cell types has been reported in mammalian species associated with different body sizes[104-106]. Additionally, the absence of variance in cell distribution could be a factor of the methodology adopted for data collection; 2D analysis of micrographs rather than 3D that may provide more precision.

Conclusion

There exist similarities and variations in the neuroanatomical features of the cerebrum of the compared rodent species (Wistar rats, guinea pigs, and rabbits). Variations were demonstrated in morphologic features and to a small extent, in the microscopic (cytoarchitectural) features of the M1 cerebrum of the species. These findings demonstrate similar ancestry in the species and, could be of benefit in the identification of suitable species as potential models for certain neuroscience research, especially investigations related to neurological conditions in human medicine. In spite of this progress, much to be learned still abounds. Thus, investigations using other approaches including immunocytochemistry, electron microscopy, and comparative neurobehavioral assessments amongst others are recommended to increase precision and further elucidate the findings of this study.

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