

Comparative Anatomy and Histology of Elephant, Human, and Tree Shrew Brains

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ABSTRACT

OBJECTIVE This study was designed to compare elephant brain morphology and histology with that of humans and tree shrews.

METHODS The size of elephant, human, and tree shrew brains were measured. Brains were sectioned and stained by the modified Mulligans for investigation of the ratios of gray matter and brain volume. The prefrontal gyrus and cerebellum were Nissl stained. The elephant cerebellum was prepared for Golgi staining.

RESULTS The gyrification of the elephant cerebrum was found to be like similar to that of the human brain, but the gyrification of the tree shrew was not determined. The ratio of gray matter and brain volume of the elephant was less than that of both human and tree shrew brains. However, the olfactory bulbs and cerebellum of both the elephant and tree shrew were large. The cortical layers I–VI of the prefrontal cortex in both elephants and humans did not show a distinct layer IV. Additionally, elephant cortical neurons were larger than those found in humans. Purkinje neurons in the elephant cerebellum were uniquely shaped, having two main dendrites extending from the cell body.

CONCLUSIONS The elephant brain gyrification is similar to the human brain. However, it has large olfactory bulbs and cerebellum as does the tree shrew brain. The cortical and Purkinje cells in elephants are larger and more dendritically branched than those of humans.

KEYWORDS comparative study, brain, elephant, human, tree shrew

INTRODUCTION

The elephant is the second largest animal found on Earth. Elephants demonstrate their intelligence in ways similar to humans. For example, they show their intelligence through painting pictures, kicking footballs, and showing compassion for members of individual groups (1).

The elephant brain is similar to the human brain as evidenced by the high number of convolutions (2,3), the ratio of brain weight at birth to the adult brain weight (2), the ratio of the volume of gray matter to the volume of total brain, the cross-sectional area of the corpus callosum (3) and the number of cortical spiny

neurons (2,4). The differences between an elephant and human brains are well-established. The gyrification of the elephant brain is more complex than that of humans and the olfactory bulb of the elephant brain is very large and contains a cavity, while the olfactory bulb of the human brain is very small (5). The temporal lobe of the elephant brain expands laterally while that of the human brain has no lateral expansion (5). Additionally, the hippocampus of an elephant brain is somewhat smaller than that of a human brain (6). Tree shrews are small and move very fast. They are closely related to primates and have been used as an alternative to primates in experimental studies of psychosocial stress (7) and secondary visual pathways (8,9). The brain to body mass ratio is thought to roughly indicate the intelligence of an animal. This ratio in tree shrews is higher than that of humans and is highest of any known animal (10). The morphology and components of the African and Indian elephant brains have been studied for decades (11). The histological features of the brains of elephants from different regions have been identified using different techniques. The morphology of the pyramidal cells of the African elephant can be clearly stained by Nissl staining and the Golgi technique (4). The cortical and cerebellar layers of the Asian elephant can be identified by hematoxylin and eosin staining (12) although staining does not reveal neuronal morphological characteristics. Comparisons of the morphological characteristics of Indian and of African elephant brains with those of humans have been reported (3,5,6). However, a comparative study of morphological and histological characteristics of the Asian elephant brain and that of human and tree shrew brains, which could provide a better understanding of the social behaviors and movement skills of the Asian elephant, has not been conducted.

The aim of this study is to compare the morphology and histology of the Asian elephant brain to that of human and tree shrew brains.

METHODS

Four adult Asian elephant brains plus one half of another elephant brain (3 males, Plai PL, and Plai D and Plai unknown and 1 female, Pung

B, all of unknown age) plus one baby elephant brain (male, one day old), 4 human brains (03-11-01 HU, 12-11-01 HU, 12-11-02 HU, and 12-11-03 HU), and 4 tree shrew brains (07-10-05 KP, AY 07-11-02, AY 07-11-04, and AY 07-11-05) were used in this study. The elephant brains were generously provided by the National Elephant Institute Lampang. The human brains were obtained from cadavers maintained by the Department of Anatomy, Faculty of Medicine, Chiang Mai University. The tree shrew brains were donated by a research project (study code: ANA-2556-01586). The present study was approved by the Ethical Review Committee for Research in Human Subjects, Faculty of Medicine, Chiang Mai University (CMU) (No. Exemption-8620/2021).

Measuring the size of the cerebrums and cerebellums

The gyrification of all brains was observed. The cerebrums and cerebellums of 3 adult elephants (Plai PL, Plai D, and Pung B), 3 humans (12-11-01 HU, 12-11-02 H, and 12-11-03 HU), one baby elephant, and 3 tree shrews (AY 07-11-02, AY 07-11-04, and AY 07-11-05) were measured for the anteroposterior (AP) length of cerebrum and cerebellum as well as the bitemporal (BT) and bi-occipital (BO) of the cerebellum in centimeters (cm) using a ruler for the elephant and human brains and a vernier caliper for the tree shrew brains. The AP length of the cerebrum was measured from the frontal to the occipital poles. The bitemporal length was measured from the most lateral side of one temporal lobe to the other. The AP length of the cerebellum was measured from the caudal surface to the rostral surface. The bi-occipital length of the cerebellum was measured between the most lateral surfaces of the cerebellum. The dimensions of the specimens was measured by an anatomist who was blinded to the donor, three times for each specimen. All data is reported as average $\pm$ standard deviation (SD).

Measuring the gray matter volume of cerebrum and hippocampal formation

The left half of the brain of a human (03-11-01 HU) and the left half brain of an elephant (unknown name) brain were sliced in a coronal

plane at a 0.7 cm thickness using a ham slicer. A whole tree shrew (TS) brain (07-10-05 KP) was sectioned in a coronal plane at a 3 mm thickness using a blade. To differentiate the gray and white matter areas of the brain slices, all brain slices were stained by the modified Mulligan's technique (13). All stained brain sections were photographed. The area of gray matter and of the total brain in all sections of the elephant, human, and TS brains and the area of the hippocampus (HP) from the 23rd - 27th sections of the elephant brain and from the 7th - 11th sections of the human brain were measured by three anatomists who were blinded to the donor, three times for each specimen, using the image J program (NIH). The volume of the gray matter, HP, and total brain volume of each section was calculated by multiplying the area by the 0.7 cm thickness. The ratio of gray matter to total brain volume of the elephant and human (half brain) and TS (whole brain), and the ratio of HP volume to the total brain volume of the elephant and human brains were calculated.

Measuring the area of corpus callosum

Three human brains (12-11-02 HU, 12-11-02 HU, and 12-11-03 HU) were mid-sagittally sectioned. The medial side of each hemisphere was photographed. The most medial sagittal section of the right half of the elephant brain (Pung B) was photographed. The Nissl-stained sagittal section of the TS brain (07-10-05 KP) containing the corpus callosum were photographed. The area of the corpus callosum of each mammal was measured by three anatomists who were blinded to the donor, three times per specimen using the Image J program (NIH). The average areas of the corpus callosum were calculated.

All of the cross-sectional area of corpus callosum data is reported as average $\pm$ SD.

Neuronal distribution in the cerebellum of elephants

To study the distribution and density of neurons in the cerebellum of humans (12-11-01 HU) and of elephants (Pung B), brain samples were sectioned using a sliding vibratome with a 50 μ m thickness then were processed for Nissl's staining to allow comparisons to be made. The density of the Purkinje cells of elephants and

human was observed and compared as well.

Morphological characteristics of elephant cerebellar neurons

To identify the morphology of the cerebellar neurons, a brain tissue block of approximately 1 cm³ was taken from an elephant brain and prepared for Golgi's staining as was done in a previous study (14).

RESULTS

The comparative morphology

The adult elephant brain is the largest and the TS brain is the smallest among these three mammals (Figure 1). The gyrification of the cerebral cortex of the elephant is relatively more complex than that of humans. There is no gyrification in the tree shrew brain. The cerebellum of elephants and TS can be seen from the dorsal view while that of the human brain is largely obscured by the occipital poles. The temporal lobes of both adult and baby elephants are laterally extended. Interestingly, the baby elephant brain has a large cerebrum and cerebellum. The olfactory bulbs of elephants and of TSs are somewhat larger than that of humans. The olfactory bulbs of both elephants and tree shrews extend anteriorly beyond the frontal poles of the cerebrum. There is a large cavity in the elephant olfactory bulb, as shown in Figure 2. However, human olfactory bulbs are very small and are in the olfactory sulci on the orbitofrontal surface of the cerebrum.

Figure 3 shows the comparative sizes of the cerebrum and cerebellum of elephants, humans, and tree shrews. As for the cerebrum, there was no difference in the anteroposterior (AP) length between an elephant, baby elephant, and human cerebrums. The AP length of the elephant cerebrum (17.6 ± 4.73 cm) was approximately 9 times that of the tree shrew's cerebrum (2.03 ± 0.15 cm). The bitemporal length of the adult elephant cerebrum (23.83 ± 3.69) was approximately two times that of humans (12.13 ± 0.61 cm). The bitemporal length of a baby elephant cerebrum (19.00 cm) was similar to the that of an adult elephant. The bitemporal length of a tree shrew (1.9 ± 0 cm) is ten times less than that of a baby elephant. As for the cerebellum, the bioccipital length was found to be longer than the AP length in all species. The AP length of

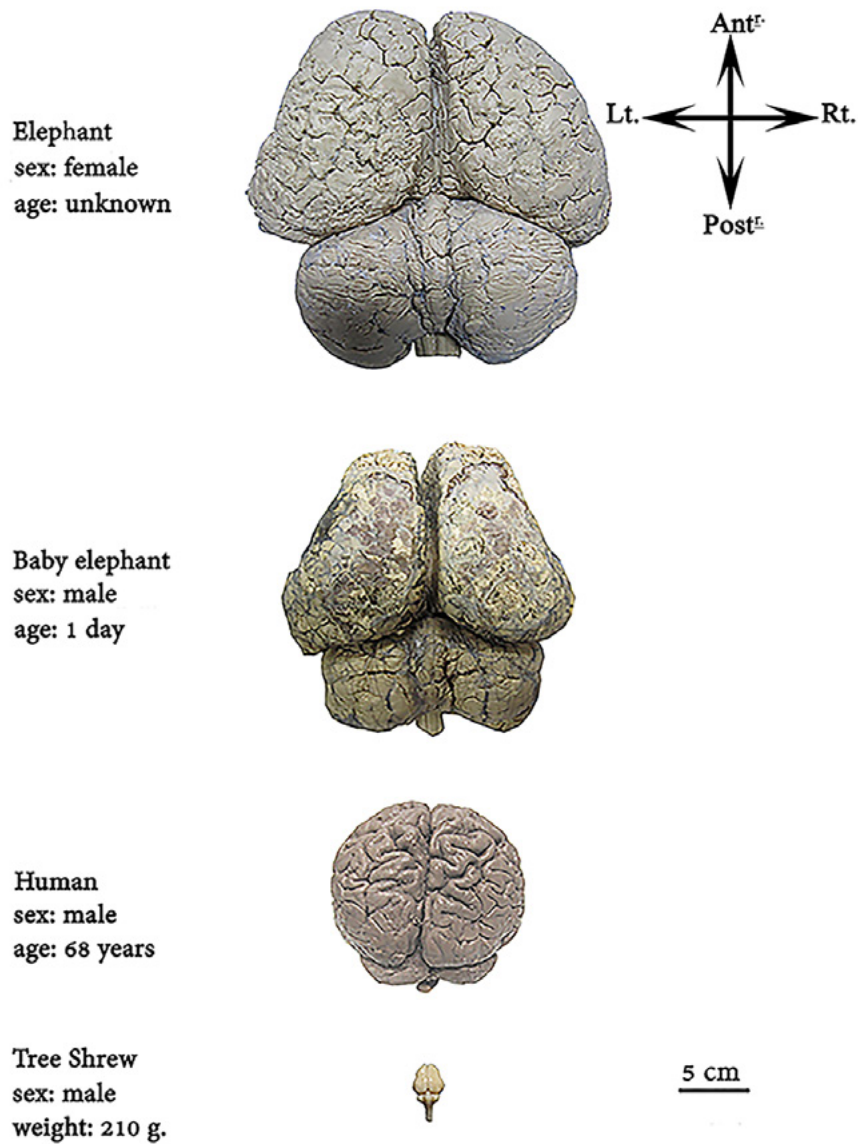


Figure 1. Dorsal views of adult (Pung B) and baby elephant brains, a human (03-11-01 HU) brain and a tree shrew (07-10-05 KP) brain. Ant^r.= anterior; Post^r.= posterior; Lt.=left and Rt.=right.

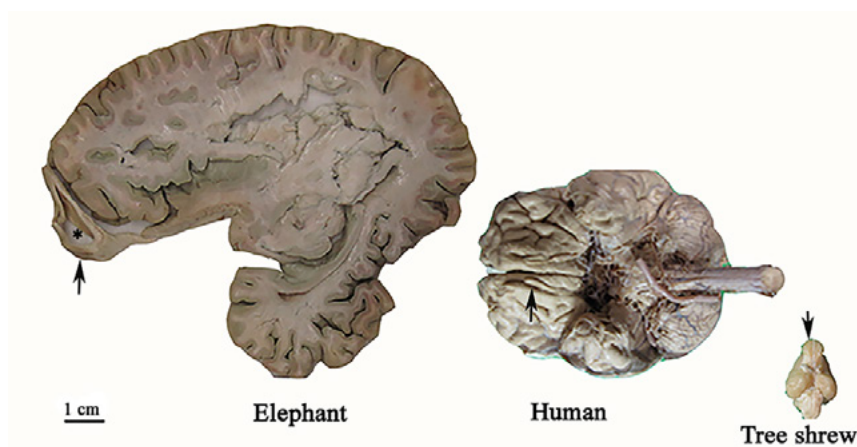


Figure 2. Sagittal section of an elephant brain showing the olfactory bulb (Pung B), ventral views of a human (12-11-01 HU) and a tree shrew (AY 07-11-02) brain. The scale is 1 cm. The black arrows point to the olfactory bulbs of all brains. * indicates a cavity in the olfactory bulb.

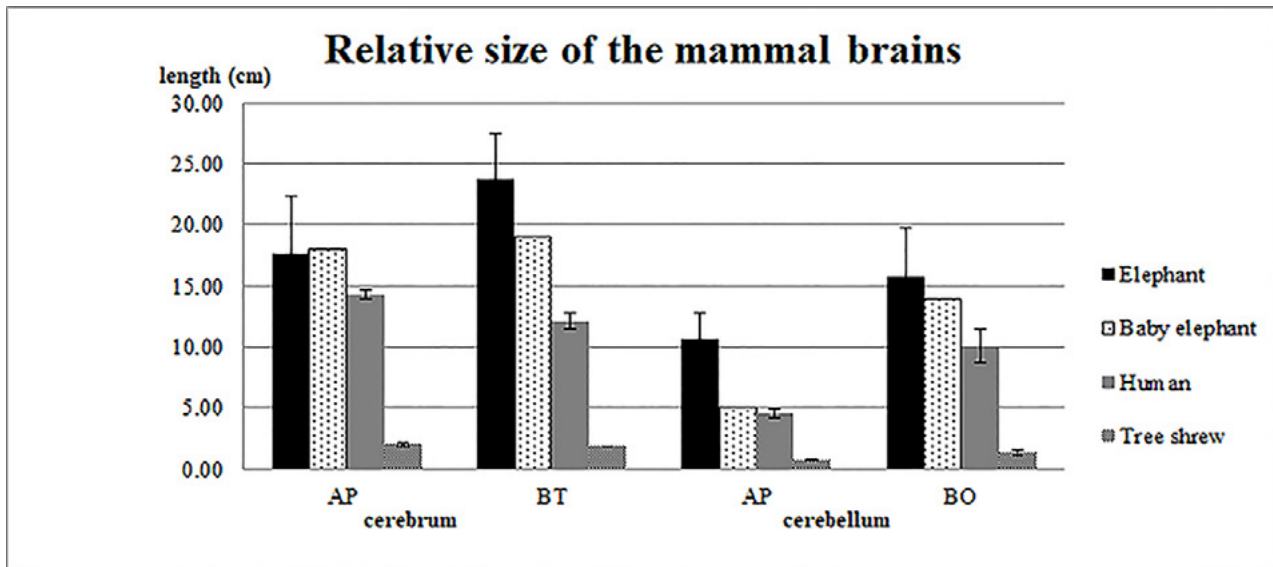


Figure 3. The anterior-posterior (AP) and bitemporal (BT) lengths of elephant cerebrums and the AP and bi-occipital (BO) lengths of elephant cerebellums comparing with those of, baby elephants, humans, and tree shrews

the elephant cerebellum (10.67 ± 2.08 cm) was larger than that of a baby elephant (5.00 cm) and that of a human (4.57 ± 0.35 cm) as well. The bioccipital length of an elephant (15.83 ± 3.88 cm) was larger than that of humans (10.13 ± 1.36 cm). However, no difference was found in the bi-occipital length between adult and baby elephants. The bi-occipital length of the tree shrews (1.37 ± 0.25 cm) was approximately one tenth that of the baby elephant.

The coronal sections of the brains

Coronal brain sections were used to measure the thickness of gray matter, deeper lobes, insular lobes, and deeper structures such as the hippocampus (HP) (Figure 4).

The gray matter in elephants was somewhat thinner than that found in humans. The white matter area of each section in elephants was somewhat larger than that of the comparable sections in humans. The insular lobe of the elephants was superficially located in the lateral fissure and its gyri were contiguous with those of the temporal lobe. On the other hand, the insular lobe of the humans was located deep on the floor of the lateral fissure. The insular lobe of the tree shrew was indistinct (Figure 4). The elephant HP was vertically oriented and formed the medial wall of the inferior horn of the lateral ventricle while the human hippocampus lay horizontally and formed the floor of the inferior horn of the lateral ventricle (Figure 4).

The ratio of gray matter and hippocampus volume to total brain volume

The total brain volume and gray matter volume of elephant and human hemispheres and that of tree shrew brains were measured and analyzed from a total of 32, 15, and 7 sections, respectively. The ratio of gray matter volume and total brain volume of elephants (0.40 ; $691.09 \text{ cm}^3/1720.33 \text{ cm}^3$) was lower than that of both humans (0.52 ; $243.41 \text{ cm}^3/464.81 \text{ cm}^3$) and tree shrews (0.48 ; $4.30 \text{ cm}^3/2.58 \text{ cm}^3$). Thus, the ratio of the gray matter volume to the total brain volume of elephants is somewhat less than that of humans and tree shrews.

We investigated the coronal sections containing the hippocampus (HP) in human and elephant brains. The HP was measured in 5 sections in both elephant and human brains. The length of the HP of both mammals were found to be approximately 3.5 cm (5×0.7 cm). The average area of elephant HP and that of humans was found to be 0.906 cm^2 and 0.688 cm^2 , respectively. Thus, the volume of elephant HP and that of humans were found to be 3.17 cm^3 ($0.906 \text{ cm}^2 \times 3.5 \text{ cm}$) and 2.41 cm^3 ($0.688 \text{ cm}^2 \times 3.5 \text{ cm}$), respectively. The ratio of hippocampus volume to the half brain of elephants and that of humans was found to be 0.002 ($3.17 \text{ cm}^3/1720.33 \text{ cm}^3$) and 0.005 ($2.41 \text{ cm}^3/464.81 \text{ cm}^3$), respectively. Therefore, the human HP is approximately 2.5 times larger than that of an elephant.

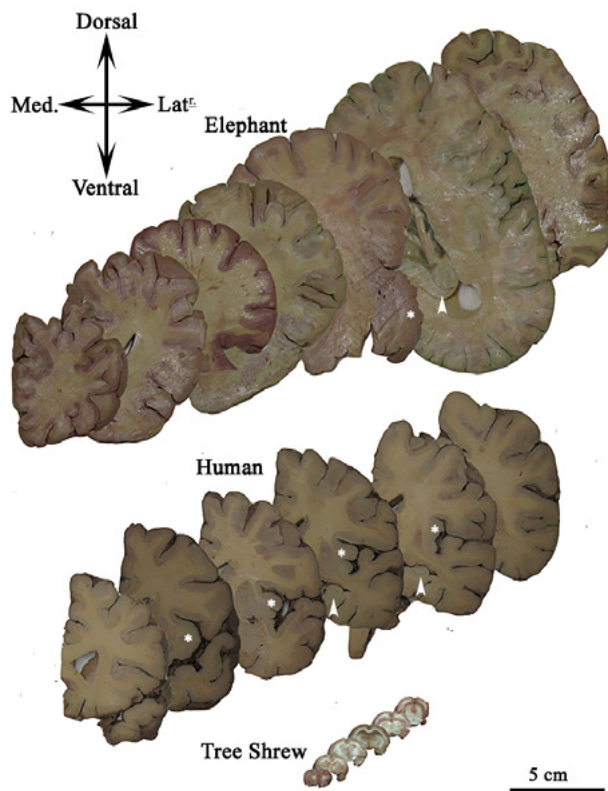


Figure 4. A series of coronal brain sections of the left hemisphere of humans (03-11-01 HU), elephants (Plai D), and the coronal brain sections of tree shrews (07-10-05 KP). The sections are arranged in order from front to back. The scale bar is 5 cm * = insular lobe, white arrowhead = Hippocampus, Med. = Medial, Lat^r. = lateral

The cross-sectional area of corpus callosum

We found that the cross-sectional area of the corpus callosum on the medial surface of the elephant hemisphere was approximately twice that of humans ($13.11 \pm 0.13 \text{ cm}^2$ vs $5.96 \pm 0.58 \text{ cm}^2$, respectively). This area in the tree shrew was 0.03 cm^2 . This suggests that there are an abundance of neuronal axons crossing the midline from one area of one hemisphere to the corresponding area on the other hemisphere of the elephant brain.

Histological comparison of the precentral gyrus and cerebellum between elephants and humans

The Nissl-stained precentral cortex of both elephant (Figure 5A) and human (Figure 5B) revealed the cortical layers I (molecular layer), II (external granule cell layer), III (external pyramidal cell layer), V (internal pyramidal cell layer), and VI (multiform layer). Neither the precentral cortex of elephants nor that of humans showed a distinct layer IV (internal granule cell layer). At the same magnification, the elephant cortex is thicker, and the cortical neurons are larger than those in humans. The cortical layer I in elephants was significantly larger than that of the human brain. Most neu-

rons in the cortical layers III and V were pyramidal cells in both species. The layer V human pyramidal cells presented a single apical dendrite projecting to the cortical surface (Figure 5B), while those of the elephant had several apical dendrites projecting in different directions (Figure 5A).

The Nissl-stained cerebellum in elephants (Figure 6A) and in humans (Figure 6B) exhibited three distinct layers of cerebellar cortex: the molecular (M), Purkinje cell (P) and granule cell (G) layers. The Purkinje cell layer was the only layer of Purkinje cells found in both species. The cell bodies of Purkinje cells of the elephant cerebellum showed a more evenly distributed arrangement than those of the humans. Interestingly, the Golgi impregnated Purkinje cells of elephants exhibited a V shape like primary dendrites extending from their cell bodies (insert in Figure 6A).

DISCUSSION

Previous studies have been done on African elephant brains (3,4,15). However, this study reported on the comparative morphology and histology of Asian elephants that had resided at the Thai Elephant Conservative Center and compared that with human and tree shrew

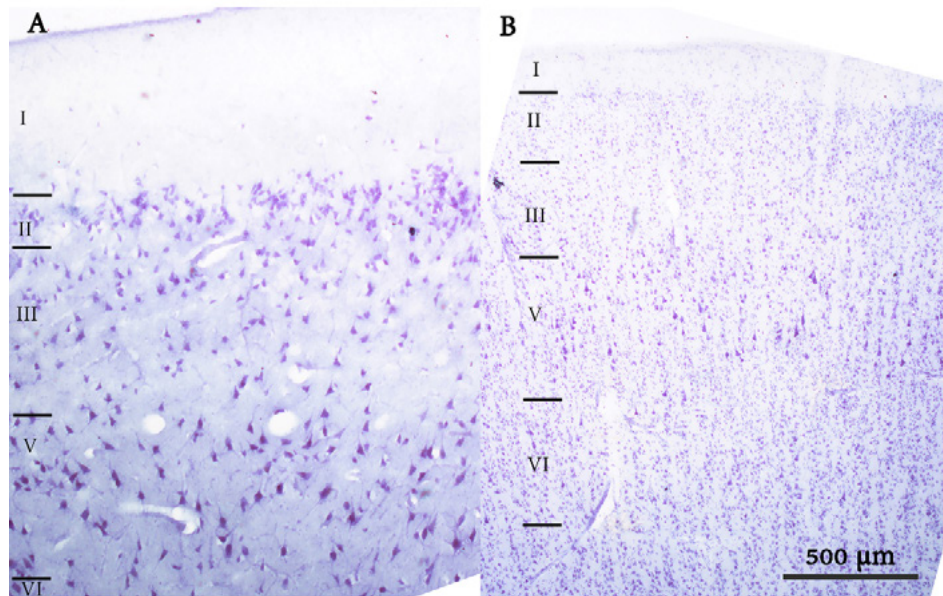


Figure 5. The Nissl-stained precentral gyrus of elephant (A) and human (B) showing that the cortical layer I–VI lacked a distinctive layer IV. The 500 µm scale bar applies to both panels A and B. I – molecular layer; II – external granule cell layer; III – external pyramidal cell layer; V – internal pyramidal cell layer; and VI – internal granule cell layer

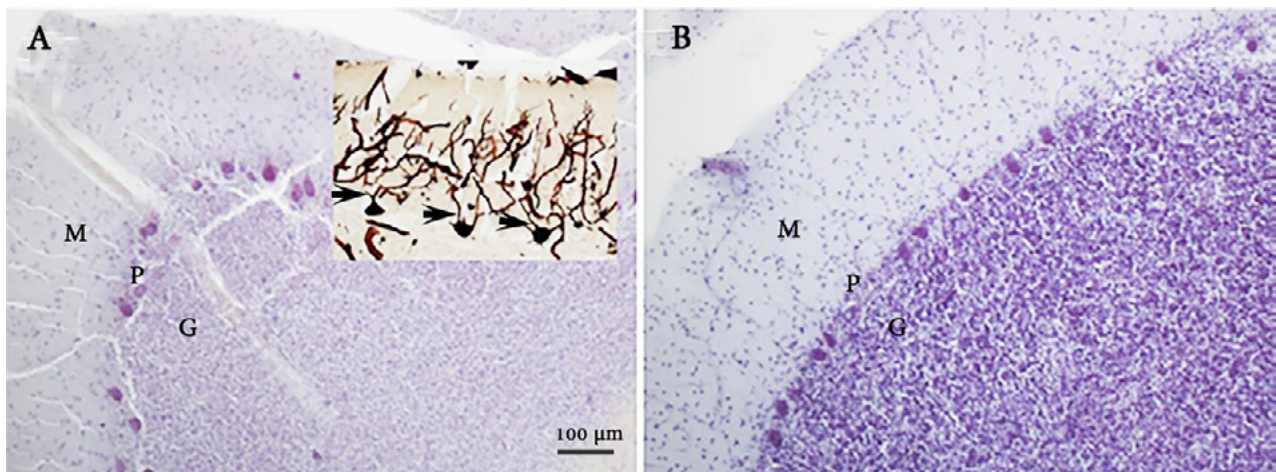


Figure 6. Nissl-stained cerebellar cortex of elephants (A) and humans (B). Golgi impregnated Purkinje cells in the elephant cerebellum are shown in (the inserted panel) in A. The black arrows in the inserted panel indicate the V-shaped dendrites. The scale bar in A is 100 µm and applies to both panels A and B.

M, molecular layer; P, Purkinje cell layer; G, the granule cell layer

brains. Our study found that the size of elephant brains was noticeably different from that of human and tree shrew brains and were related to their body sizes (5), as well. The tree shrew did not have gyrification like elephant and human brains do. Based on their smaller and less complex brains, tree shrews are likely more primitive than elephants and humans (16). Even though there is no gyrification on tree shrew brains, the brain-to-body mass ratio of tree shrews is the highest of all mammals (10). All three of the mammals in this study

have good cognitive functioning and are intelligent. Large olfactory bulbs are found in both elephants and tree shrews, while small olfactory bulbs are found in humans. The large olfactory bulb in both elephants and tree shrews reflects the behavioral aspects of these animals for living and mating (5). The elephant olfactory bulbs contained cavities or ventricles which is consistent with findings of previous studies (5). The human occipital poles containing primary visual areas are more prominent than those of both elephants and tree shrews. The

relative importance of functions related to olfaction and vision in elephants and humans are in opposite directions (16). Both elephants and tree shrews demonstrated relatively large olfactory bulbs and non-prominent occipital poles. Because of its large eyes and large superior colliculi, the tree shrew is a good model for studying the secondary visual pathway which is involved in detecting moving objects (8,9).

In addition to large olfactory bulbs, a large cerebellum was also found in both elephants and tree shrews. In this study, the cerebellar AP length of the elephant was found to be approximately two times that of the human cerebellum. Previous studies have reported that the weight of the elephant cerebellum is 1.8 times that of the human cerebellum (5). The relative size of the elephant cerebellum is the largest among the mammals (17,18) and contains 97.5% of the total neurons found in the brain (15). Because they have a large cerebellum which contains a higher number of cerebellar neurons than other mammals, elephants demonstrate high levels of sensorimotor functioning. Tree shrews are fast moving animals, so their large cerebellum might be related to efficient movement skills, as well. This study demonstrated that elephant Golgi-stained Purkinje cells have two primary dendrites which extend from the soma and branch out to the molecular layer, a finding which is consistent with previous studies (18). These Purkinje cells are arranged in one layer and have a wider gap than those in humans. These dendritic morphologies and the intervals between the Purkinje cells reflect the complexity of dendrites and allow the climbing fibers to pass through that layer to make synapses on dendrites (18). In this study, the cerebellar AP length in baby elephants was approximately half that of adult elephants. This might reflect a progressive development of the cerebellum in the AP direction during an immature stage. The developing cerebellar lobes might include vermis, paravermis, and cerebellar hemispheres which respond in well-developed spinocerebellar and cerebrocerebellar functions in elephants.

Our coronal sections revealed a more outward extending insular lobe and a relatively smaller hippocampus with a more vertical orientation

in elephants than in humans which is consistent with a previous study (5). The outward part of the insular lobe is found in elephants because of the lateral expansion of the temporal lobe (13). This may reflect the well-developed auditory system in elephants. Because of its large cerebrum, the elephant HP is relatively small compared to that of humans (6). However, an MRI study of elephant brains demonstrated a large and highly convoluted HP resulting in long-term memory in elephants (3). The cross-sectional area of the corpus callosum in elephants was approximately two times larger than in humans. The elephant corpus callosum is the largest among mammals, having a weight of 5,000 gm (19).

In this study, the gray matter to brain volume ratio in Asian elephants was 0.40 which is close to the ratio found in African elephants, 0.35 (3). This ratio in elephants is lower than in humans and tree shrews. The histology of the precentral gyrus in elephants demonstrated a lack of a distinctive cortical layer IV, which is an internal granular layer, a finding also reported in a previous study (4). The layer IV of human precentral gyrus or premotor cortex was barely discernable in this study. A thin layer IV in humans might result from receiving little sensory information from the thalamus (20). The pyramidal cells in the elephant precentral gyrus were found to be larger and their apical dendrites project in different directions from those of humans. A previous study also reported that elephant pyramidal cells are larger than those of primates and differ in morphology/orientation (4). This study found smaller cortical cells in humans than in other mammals. Spiny cortical neurons were found in both elephants and human cortexes (2,4). The spiny and diverted dendritic neurons, especially in the precentral gyrus, might allow inputs from the cerebellum and basal ganglia because of the abundance of synapses on these cortical dendrites. Our study also found wide dendritic branches of Purkinje cells which receive spinal, vestibular, and cerebral inputs. Movement skills or functions in elephants and humans require indirect connections between the cerebellum and the premotor cortex. A previous study reported higher numbers of cortical neurons in humans than

in elephants, resulting in higher human brain function compared to elephants (16). It has been reported that humans demonstrate outstanding intelligence compared to other primates, e.g., the ability to laugh, to imitate, and to form and interpret languages (2).

CONCLUSIONS

The elephant brain has features which are similar to human brains, e.g., high gyrification, and some which are similar to tree shrew brains, e.g., olfactory bulbs and cerebellum. The cortical layers I–VI of the precentral gyrus in both elephants and humans lack a distinct layer IV. Elephant cortical neurons are larger and have more dendritic processes extending from the soma than those of humans. Elephant Purkinje cells widely extend V-shaped primary dendrites from the cell body. The pyramidal and Purkinje neurons in elephants used for more functions than in humans.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ADDITIONAL INFORMATION

Author Contributions

N.P. performed the experiments, analyzed the data, prepared the figures, authored or reviewed drafts of the paper, and approved the final draft.

K.S. performed the experiments, analyzed the data, prepared the figures, and graphs, authored or reviewed drafts of the paper, and approved the final draft.

C.S. analyzed the data, contributed materials, authored or reviewed drafts of the paper, and approved the final draft.

T.A. analyzed the data, contributed materials, authored or reviewed drafts of the paper, and approved the final draft.

A.S. performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

S.M. performed the experiments, analyzed the data, prepared figures, authored or reviewed drafts of the paper, and approved the final draft.

C.T. conceived of and designed the experiments, analyzed the data, contributed reagents and materials, authored or reviewed drafts of the paper, and approved the final draft.

R.Q. conceived of and designed the experiments, analyzed the data, prepared figures and/or tables, prepared the manuscript, authored or reviewed drafts of the paper, and approved the final draft.

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