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Age-dependent Morphometric Changes in the Brains of Albino Rats (*Rattus norvegicus*)

Amira M. Mokhtar[1]*, Said A. Hassan[1], Eid A. Moussa[2], Tarek O. El Mahdy[1]

[1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

[2]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Arish University, Egypt.

***Correspondence**
Corresponding author: Amira M. Mokhtar
E-mail address: princesselnahla@yahoo.com

Abstract

The present study had been established to deliver an anatomical atlas for all veterinary anatomists where various structures of the rat brain through all views are shown clearly. On the other hand, the effect of both aging and sex were put into consideration through the current study. Thirty-four apparently healthy rats of both sexes (19 males and 15 females) were used in the study. The animals were classified into four groups according to their age. Cross sections of the brain were performed to document the cerebral dimensions including the cerebral length, width, and height in addition to the transverse diameter of the thalami and the thickness of the corpus callosum. Ageing would result in a negative impact on some brain structures. However, some others had not been affected as much. Furthermore, sex of the animal presented an important role as a variant where male rats of each age group demonstrated different results than their analogous female rats. Findings of this work might be used as a tool for studying the gross morphometric changes that occurred in the rat brain due to normal aging process in either sex.

KEYWORDS
Ageing, Brain, Morphometry, Rats, Sex

INTRODUCTION

'Normal aging' refers to age-related changes in the brain that occur independent of brain disorders (Burgmans *et al*., 2009; Sperling, 2011; de Flores *et al*., 2015). Aging has an impact on the gross morphology, vasculature, and neurons of the human brain, as well as cognition. Thus, aging would lead to a decrease in the brain's volume, particularly in the frontal cortex. Moreover, the possibility of brain stroke and ischemia increases with age due to aging vasculature and rise of blood pressure, in addition to development of white matter lesions (Peters, 2006). The author added that memory decline also occurs with ageing and brain activation becomes more bilateral for memory tasks to compensate the non- functioning areas.

Many age-related changes were also characteristic to neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases. Furthermore, the primary functional-structural study revealed that the inferior frontal gyrus is differentially affected by aging, however, the secondary structural studies implicated other frontal cortex regions. Remarkably, both the functional-structural study and the secondary structural studies suggest that the entorhinal cortex, a region most vulnerable to AD, turned out to be the region most resistant to normal aging (Amaducci and Tesco, 1994).

Moreover, the work also monitored brain macromorphometric changes during ageing in albino rats as a suitable animal model to study the mechanisms of aging and also for further studies and observations on aging. Also, detailed- anatomy of the rat brain structures is of great importance for neuroscientists.

Based on these items, it was possible to establish the criteria of evaluation of the morpho-functional characteristics and the physiological status of brain, a highly specified component of the nervous system and morphologically very sensitive to aging process.

MATERIALS AND METHODS

Experimental animals

The present work was carried out on 34 apparently healthy albino rats of both sexes (with known dates of birth). The rats were obtained from the laboratory animal house, Suez Canal University. The animals were kept at room temperature with normal humidity and ad libitum access to food and water. The Suez Canal University Laboratory Animal Care and Use Committee approved all animal experiments.

Morphometric evaluation

A total of 34 rats (19 males and 15 females) were used to investigate the various brain dimensions at different ages starting from three months up to 24 months old. Each animal was euthanized by an overdose of chloroform in a closed container. Then, the heads of the animals were cut off at the atlanto-occipital articulation and the brains were removed from the cranial bones of the skull, followed by an immersion in 10% neutral buffered

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formalin saline for 24 hours.

The brains of all rats were used to measure various cerebral dimensions and some other brain structures. The animals were divided into the following groups: G1: Juvenile rats aged 3 months (4 males and 7 females). G2: Young rats aged 6-7 months (3 males and 4 females). G3: Middle-aged rats aged 10-13 months (8 males and 2 females). G4: Late-aged rats aged 19-24 months (4 males and 2 females).

The length, width, and height of the cerebral hemispheres were accurately measured (Figs. 2 and 3). Then, the brains were put on a level surface and cross sections were taken as accurately as possible and perpendicular to the longitudinal axis of the brain. All cut sections were made at three levels on the ventral surface of the brain (Fig. 1). The obtained cuts revealed the rostral face of each level. The thickness of the corpus callosum and transverse diameter of the thalami were all accurately measured as well (Fig. 3). The previous technique for linear morphometric measurements of the rat's brain was adopted according to Duffell *et al.* (2000).

The various views of the brain (dorsal, lateral, and ventral) and the brain cut sections were all photographed using a digital camera (Nikon, CoolPIX, L100, Japan).

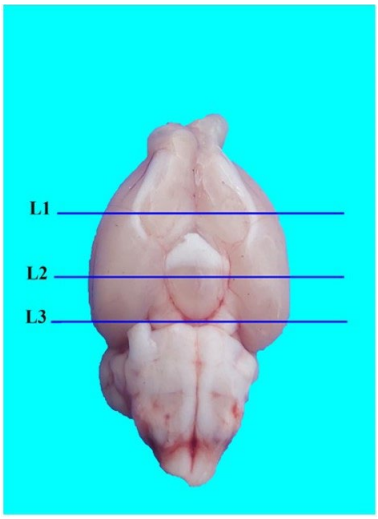

Fig. 1. A photograph of 3 months old rat's brain (ventral view) showing the different levels of the cross sections taken.

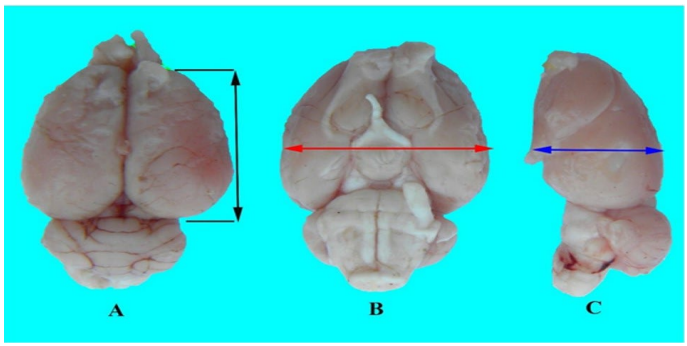

Fig. 2. A) The cerebral length (black arrow heads) was measured from the frontal pole (i.e. most rostral extremity of the frontal lobe) to the occipital pole (i.e. most caudal extremity of the occipital lobe). B) The cerebral width (red arrow heads) was measured at a level passing through the mid-distance between the two poles of the cerebrum (level of the tuber cinereum). C) The cerebral height (blue arrow heads) was measured at the mid-point between the two poles of the cerebrum along the lateral aspect of the brain (level of the tuber cinereum).

Nomenclature

The nomenclature used in this study was adopted according to the Nomina Anatomica Veterinaria (NAV, 2017) whenever needed.

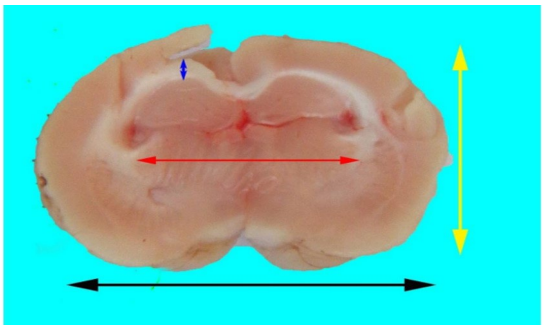

Fig. 3. A cross section of 3 months old rat's brain at the level of the tuber cinereum showing the points of measurement of brain structures.

RESULTS

The brains of rats of all age stages through linear morphometric results revealed significant statistical differences between individuals of the same sex, and definitely between males and females of same age stage.

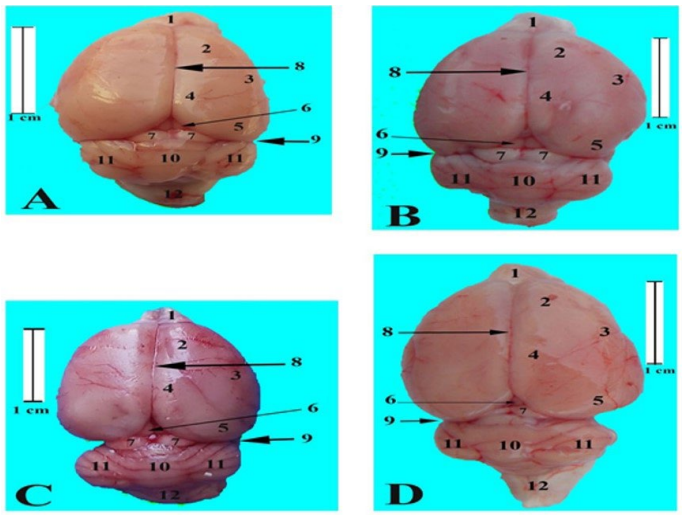

Fig. 4. A photograph of the rat's brain (dorsal view).

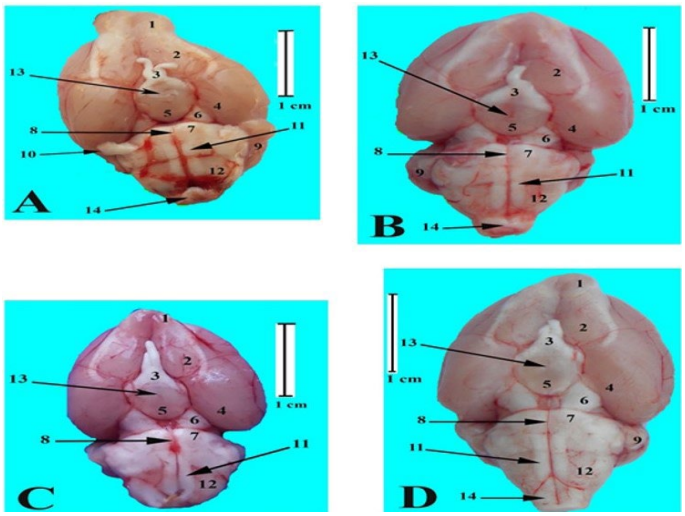

Fig. 5. A photograph of the rat's brain (ventral view).

The obtained results clarified that male rats, in general, presented significantly larger dimensions ($P < 0.05$) compared to female rats of the same age, indicating that the sex factor had an

impact on the dimensions of most brain structures.

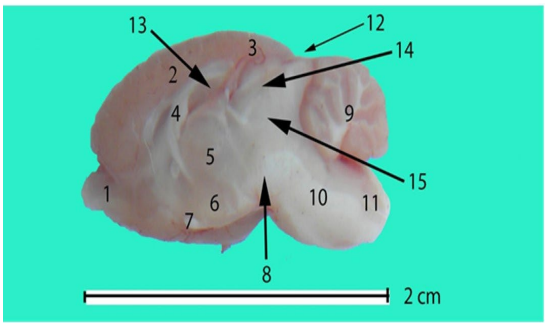

Fig. 6. A photograph of the brain of 3 months old female rat (medial surface of the right half).

The cerebral dimensions were a noticeable marker of aging where the cerebral length was increased in male rats from 3 to 6 months, followed by a decline in 10 months and a more decrease in 24 months old rats.

Female rats followed the same pattern as males, where the increase in juvenile and young animals was encountered, however, the decrease in the adult and late-aged was much stronger than that of analogous males indicating the effect of aging was much tremendous.

Surprisingly, there was not any significant difference of the cerebral length at various age groups whether in male or female animals (Fig.7A and Table 1).

Table 1. Cerebral length (Mean ± SD) /cm

Age group	Male	Female
G1	1.58 ± 0.15	1.58 ± 0.07
G2	1.68 ± 0.10	1.63 ± 0.03
G3	1.66 ± 0.09	1.52 ± 0.15
G4	1.57 ± 0.07	1.44 ± 0.04

On the contrary, the cerebral width increased through aging in males and females. Three-months old male rats were significantly lower than that of the other groups. The cerebral width dimensions were higher in females of the first and third groups, but lower in the second and fourth groups compared to the males of the same age. The previous records did not achieve any significant result (Fig.7B and Table 2).

Table 2. Cerebral width (Mean ± SD) /cm.

Age group	Male	Female
G1	1.46 ± 0.08	1.49 ± 0.06
G2	1.56 ± 0.14	1.53 ± 0.11
G3	1.58 ± 0.07	1.59 ± 0.07
G4	1.62 ± 0.03	1.59 ± 0.03

Cerebral height in males was the highest in the second stage, while the first and third age stages did not differ too much, followed by a mild increase in the fourth stage. These results did not show any significant difference.

Cerebral height in females was slightly higher in the first stage when compared to the second stage, followed by a significant increase ($P < 0.05$) in the third and fourth stages. The latter stages showed a relatively constant rate of growth.

Thus, the cerebral height dimensions were higher in males than females in 3 and 6 months old rats and higher in females than males in 10 and 24 months old rats (Fig.7C and Table 3).

The study highlighted the morphometric measurements of the thalami and corpus callosum. These brain structures represented remarkable evidence of the changes due to normal aging process.

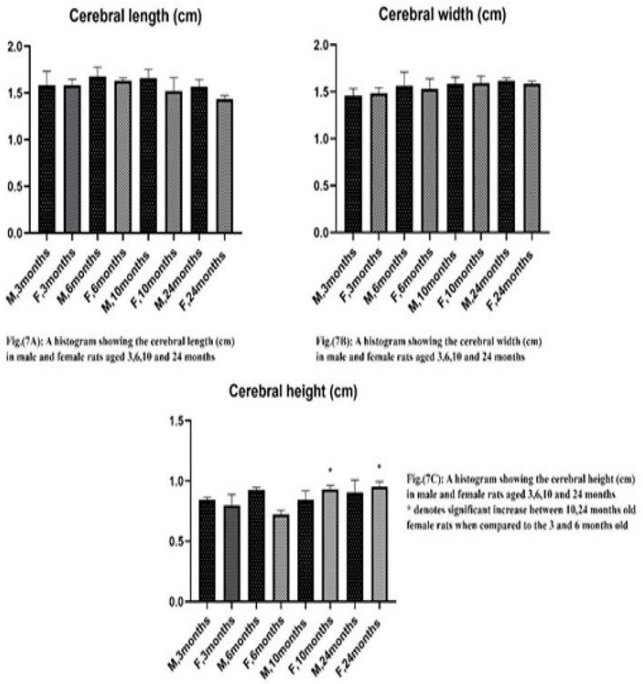

Fig. 7. A photograph of histograms showing the cerebral length, width and height (cm).

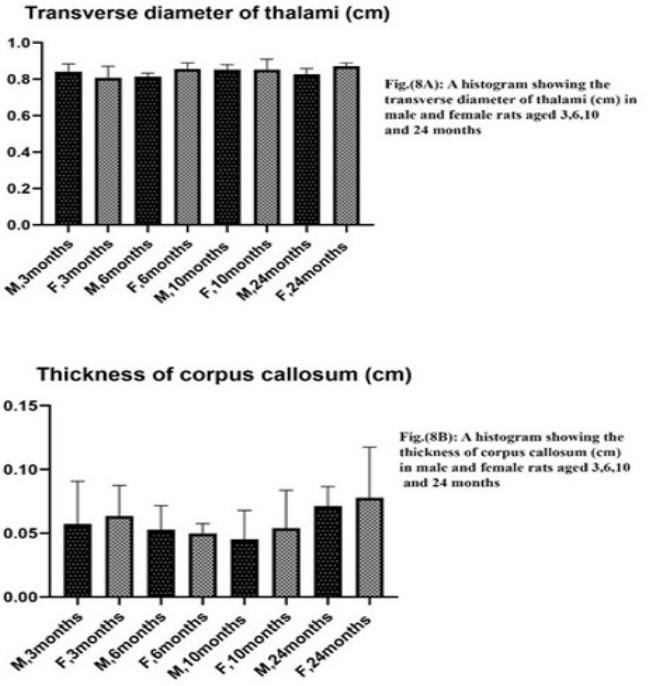

Fig. 8. A photograph of histograms showing the transverse diameter of thalami and the thickness of corpus callosum (cm).

Transverse diameter of the thalami showed fluctuating records through aging, where it was slightly higher in males of the first group than that of the second group, whereas such dimension of the third group was insignificantly higher than the fourth group. However, the fourth group presented a thalamic diameter

being insignificantly lower than that of the first group. On the other hand, female rats of different ages showed the opposite fluctuating records when compared to males.

Table 3. Cerebral height (Mean ± SD) /cm.

Age group	Male	Female
G1	0.85 ± 0.02	0.80 ± 0.09
G2	0.93 ± 0.02	0.72 ± 0.03
G3	0.84 ± 0.07	0.93 ± 0.03
G4	0.91 ± 0.10	0.95 ± 0.04

The transverse thalamic dimensions in male and female subjects of the third age group were approximately the same. In 3 months old, the thalamic diameter in males was higher when compared to analogous females. Later, 6 and 24 months old female rats had higher thalamic diameter than males. However, these records did not achieve any significant result whether in male or female rats (Fig. 8A and Table 4).

Table 4. Transverse diameter of the thalami (Mean ± SD) /cm.

Age group	Male	Female
G1	0.84 ± 0.04	0.81 ± 0.06
G2	0.83 ± 0.02	0.86 ± 0.03
G3	0.85 ± 0.03	0.85 ± 0.06
G4	0.83 ± 0.03	0.87 ± 0.02

The thickness of the corpus callosum reached the highest record in male and female rats of 24 months old. However, the other three groups presented decreaesd value of the thickness in 3,6 and 10 months old male rats.

In addition, female rats of 3,10, and 24 months old showed an increase in the corpus callosum thickness and the lowest value was recorded in 6 months old ones.

These measurements showed that females had a thicker corpus callosum when compared to males of the same age groups, except in 6 months old age group where males had a slightly higher thickness. The obtained values did not achieve any statistical significance whether in male or female rats (Fig. 8B and Table 5).

Table 5. Thickness of the corpus callosum (Mean ± SD) /cm.

Age group	Male	Female
G1	0.057 ± 0.034	0.063 ± 0.024
G2	0.053 ± 0.019	0.050 ± 0.008
G3	0.045 ± 0.023	0.054 ± 0.030
G4	0.072 ± 0.015	0.078 ± 0.040

DISCUSSION

The key to understand the morphometrical changes which occurred through our study was Image-J " software, that explained the impact of normal ageing process on the brain and brain structures of albino rats of both sexes.

These linear morphometric parameters appeared simple and easily performed, but they might be evaluation criteria for the growth and development of the rat's brain in young, middle-aged, and late-aged animals.

It was observed here that the thickness of the fibers of the corpus callosum reached their maximum values at 24 months old in both sexes. However, the values of the corpus callosum thickness were fairly greater in female subjects than their male analogous nearly through the studied age groups, particularly in middle-aged and late-aged animals. However, Duffell *et al*. (2000) recorded the maximum corpus callosum thickness in Wistar rats on 14 days old and nearly halved in thickness on 20 days old.

Also, there were unsignificant differences between the transverse diameter of the thalami in both sexes and the recorded values were more or less constant during ageing. Such observations indicated that aging might not affect the thalamic dimensions.

Furthermore, our results revealed a gradual increase in the cerebral length in 3 and 6 months old rats, followed by a slight decrease till reaching the minimal length in old rats aged 24 months of both sexes.

The recorded data of the cerebral width were increased through aging in both male and female animals. At the same time, the cerebral height was maximum on 24 months old of both sexes.

These findings confirmed those obtained by Duffell *et al*. (2000) who stated that most brain measurements in Wistar rats increased with age.

CONCLUSION

It was important to point out that the sex factor did not influence the various cerebral dimensions, except for the cerebral length which significantly increased in male rats when compared to female ones, especially in middle-aged and late-aged animals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Amaducci, L., Tesco, G., 1994. Aging as a major risk for degenerative diseases of the central nervous system, Current Opinion in Neurology 7, 283-286.

Burgmans, S., Van Boxtel, M.P.J., Vuurman, E.P.M., 2009. The prevalence of cortical gray matter atrophy may be overestimated in the healthy aging brain. Neuropsychology 23, 541.

de Flores, R., La Joie, R., Che´ telat, G., 2015. Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. Neuroscience 309, 29–50.

Duffell, S.J., Soames, A.R., Gunby, S., 2000: Morphometric Analysis of the Developing Rat Brain, Toxicologic Pathology 28, 157-163.

Nomina Anatomica Veterinaria (NAV), 2017. Prepared by the International Committee on Veterinary Gross Anatomical Nomenclature (ICVGAN), 6th ed. Published by the Editorial Committee. Hanover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de janeiro (Brazil).

Peters, R., 2006. Ageing and the brain. Postgrad Med J. 82, 84-88.

Sperling, R.A., 2011. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association work groups on diagnostic guidelines for Alzheimer's disease. Alzheimer's and Dementia 7, 280–292.