Draft version July 28, 2025
Typeset using LaTeX **twocolumn** style in AASTeX631

Brain Structural Preservation in Long-Lived Bats: An Epigenetic Investigation of textitRousettus aegyptiacus

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[1] *Anthropic, Gemini & OpenAI servers. Planet Earth.*

ABSTRACT

Age-related cognitive decline and brain atrophy are hallmarks of mammalian aging, yet long-lived species like the Egyptian fruit bat (*Rousettus aegyptiacus*) exhibit exceptional resilience. This study investigated the neurobiological and epigenetic mechanisms underlying healthy brain aging by examining the interplay between epigenetic age, global brain structural integrity, and spatial memory performance. We leveraged a multi-modal dataset from 41 bats, encompassing DNA methylation-based epigenetic age, total brain volume derived from Diffusion Tensor Imaging, and detailed behavioral metrics from a spatial foraging paradigm. Due to unforeseen data processing challenges, the analysis of cognitive metrics was not feasible for this report. Consequently, the study focused solely on the relationship between epigenetic age and total brain volume (TBV) in a subset of 33 bats with complete imaging and epigenetic data, employing Ordinary Least Squares regression while controlling for sex and origin colony. Our analysis revealed no statistically significant association between epigenetic age and TBV (= 0.0073, p = 0.968). This preliminary finding suggests that *Rousettus aegyptiacus* may exhibit remarkable preservation of global brain structure into advanced epigenetic age, potentially indicating a slower rate of age-related brain atrophy compared to other mammals. While these results offer novel insights into mechanisms of healthy brain aging, they should be interpreted with caution due to limitations including unanalyzed cognitive data and violations of statistical assumptions in the regression model, underscoring the critical need for future comprehensive investigations.

Keywords: Computational methods, Linear regression, Astronomy software, Normal distribution, Astronomy data modeling

1. INTRODUCTION

Aging is a universal biological process characterized by a progressive decline in physiological functions, an increased susceptibility to disease, and ultimately, mortality. Within the mammalian kingdom, a prominent hallmark of aging is the deterioration of brain health, which manifests as age-related cognitive decline and significant structural atrophy. These changes profoundly impact an individual's quality of life and represent a major public health challenge globally. Despite extensive research, the fundamental neurobiological and epigenetic mechanisms that underpin healthy brain aging, and conversely, lead to age-related neurodegeneration, remain incompletely understood. The complexity arises from the multifaceted nature of brain aging, involving intricate genetic, environmental, and lifestyle factors, making it difficult to isolate the key drivers of resilience or vulnerability. A critical gap in our current knowledge pertains to identifying species that exhibit exceptional resilience to these age-related brain changes and elucidating the molecular and structural adaptations that confer such protection.

Long-lived species offer unique and powerful opportunities to dissect the mechanisms of healthy aging, as they naturally maintain physiological integrity for extended periods. The Egyptian fruit bat, *Rousettus aegyptiacus*, stands out as an extraordinary mammalian model in this regard. With a maximum lifespan significantly exceeding that predicted by its body size, *R. aegyptiacus* exhibits remarkable resistance to various age-related pathologies, including those affecting the brain. This exceptional longevity and apparent resilience suggest that these bats may possess novel mechanisms for preserving brain structural integrity and cognitive function into advanced ages, thereby providing a powerful comparative framework to understand deviations from typical mammalian aging trajectories observed in other mammals.

To address this gap, our overarching research program endeavors to comprehensively investigate the neu-

robiological and epigenetic mechanisms underlying exceptional cognitive resilience in aging *Rousettus aegyptiacus*. This multi-modal approach integrates advanced neuroimaging techniques, precise behavioral assessments of spatial memory, and cutting-edge epigenetic analyses. Specifically, we aim to unravel the intricate interplay between DNA methylation-based epigenetic age, global brain structural integrity as measured by total brain volume, and detailed spatial memory performance. Epigenetic clocks, derived from DNA methylation patterns, offer highly accurate biological age estimates that can deviate from chronological age, potentially reflecting an individual's true biological aging rate. Concurrently, neuroimaging provides direct measures of brain structure, while behavioral paradigms allow for the quantification of cognitive function, offering a holistic view of brain health.

In this particular study, we focus on a subset of these broader objectives, specifically examining the relationship between epigenetic age and global brain structural integrity. We hypothesize that *R. aegyptiacus* exhibits remarkable preservation of global brain structure, implying a slower rate of age-related brain volume loss compared to typical mammalian aging, even into advanced epigenetic ages. To test this, we leveraged a unique multi-modal dataset from a cohort of Egyptian fruit bats, encompassing DNA methylation-based epigenetic age and total brain volume derived from Diffusion Tensor Imaging (DTI) scans. We employed Ordinary Least Squares regression to rigorously examine the relationship between epigenetic age and total brain volume, while carefully controlling for potential confounding factors such as sex and origin colony. Although our broader research program also includes an in-depth analysis of spatial memory performance, unforeseen data processing challenges precluded the inclusion of cognitive metrics in this specific report. Nevertheless, the findings presented herein contribute novel insights into the structural aspects of healthy brain aging and provide a foundational step towards understanding the fundamental mechanisms that enable exceptional brain structural preservation in a uniquely long-lived mammalian species.

2. METHODS

2.1. *Subject Cohort*

The study utilized a cohort of 41 Egyptian fruit bats (*Rousettus aegyptiacus*), sourced from two distinct origin colonies: Aseret (n=24) and Herzeliya (n=17). The cohort comprised 18 females and 23 males, providing a balanced representation for assessing potential sex-specific effects on brain aging. All bats were housed under controlled environmental conditions and provided *ad libitum* access to food and water. For the primary analyses detailed in this report, a subset of 33 bats with complete DNA methylation-based epigenetic age and Diffusion Tensor Imaging (DTI) data was used, ensuring the robustness of the investigated relationships.

2.2. *Data Acquisition and Processing*

A multi-modal dataset was compiled for each subject, encompassing DNA methylation profiling for epigenetic age determination, detailed behavioral assessments of spatial memory, and neuroimaging data for brain structural quantification.

2.2.1. *Epigenetic Age Determination*

Epigenetic age for each bat was determined using a previously established and validated DNA methylation (DNAm) clock specific to *Rousettus aegyptiacus*. DNA was extracted from skin tissue samples obtained from each bat. High-throughput DNA methylation profiling was performed, and the resulting methylation patterns at specific CpG sites were used as input for the bat-specific epigenetic clock algorithm. The output, referred to as 'DNAm_Age' (or epigenetic age), provides a biologically relevant estimate of age that can deviate from chronological age, offering a more dynamic measure of the aging process. The 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' values were directly utilized from the provided metadata.

2.2.2. *Behavioral Assessment of Spatial Memory*

Spatial memory performance was assessed using a standardized spatial foraging paradigm designed for bats. This paradigm involved a series of tests ('test1', 'test2', 'test3') designed to probe different aspects of spatial learning and memory. Data for each bat's performance was recorded in individual Excel files. For each test phase, the following metrics were extracted or calculated:

- **Latency to First Correct Entry:** Measured in seconds, this metric quantifies the time taken from the start of a trial until the bat's first entry into the correct target box. If a bat never entered the correct box within the trial duration, this value was recorded as a missing value (NaN).
- **Total Incorrect Entries:** This count represents the number of times a bat entered an incorrect box during a given test phase.
- **Total Entries:** This metric represents the total number of all box entry events (correct or incorrect) within a test phase.

- **Perseverative Errors:** Two specific perseverative error metrics were derived:
  - **Short-Term Memory (STM) Error:** A binary variable (0 or 1) derived from 'test2'. An STM error (1) was recorded if the bat's very first box entry in 'test2' was to the box that was correct in the preceding 'test1' phase, indicating a failure to update spatial memory.
  - **Long-Term Memory (LTM) Error:** A binary variable (0 or 1) derived from 'test3'. An LTM error (1) was recorded if the bat's very first box entry in 'test3' was to the box that was correct in the preceding 'test2' phase, indicating interference from prior learning.

Data extraction involved loading specific sheets ('test1', 'test2', 'test3') from each bat's Excel file, filtering for 'Box entry' ('E') or 'Box entry and took food' ('F') actions, and referencing the correct box number for each phase. Raw filenames were normalized to match subject IDs for accurate data aggregation.

2.2.3. *Brain Structural Imaging and Volume Quantification*

Global brain structural integrity was quantified through Total Brain Volume (TBV) measurements derived from Diffusion Tensor Imaging (DTI) data. DTI scans were acquired for each bat, and the raw imaging data were stored as 4D NIfTI files. The procedure for TBV calculation involved the following steps:

1. **Data Loading:** Each 4D DTI NIfTI file was loaded using the 'nibabel' library.
2. **B0 Image Extraction and Averaging:** The first three volumes of the 4D DTI dataset, which correspond to B0 images (b-value = 0, representing non-diffusion-weighted images), were extracted. These three B0 images were then averaged along the time dimension to create a single, higher signal-to-noise 3D B0 image. This averaged B0 image served as the basis for volume calculation.
3. **Voxel Volume Calculation:** The dimensions of a single voxel (in mm) were extracted from the NIfTI file's header ('.header.get_zooms()'). The volume of one voxel (in mm^3) was then calculated by multiplying these three dimensions (e.g., width $\times$ height $\times$ depth).
4. **Total Brain Volume Calculation:** The DTI data provided were already skull-stripped, meaning non-brain tissues had been removed, and their corresponding voxels had an intensity value of zero. Therefore, TBV was calculated by counting the number of non-zero voxels in the averaged 3D B0 image and multiplying this count by the previously calculated voxel volume. This yielded the Total Brain Volume in cubic millimeters (mm^3).

NIfTI filenames were normalized using the same logic applied to behavioral data to ensure accurate matching with subject IDs.

2.2.4. *Data Integration*

All processed data streams—subject metadata (including epigenetic age, sex, and origin colony), calculated behavioral metrics, and derived total brain volumes—were consolidated into a single master pandas DataFrame. A 'Normalized_ID' column, created by standardizing 'Sample_ID's and filenames across all data sources, served as the primary key for merging. This master dataset formed the basis for all subsequent analyses. The final dataset for analysis of the relationship between epigenetic age and total brain volume comprised 33 subjects with complete data.

2.3. *Exploratory Data Analysis (EDA)*

Prior to formal statistical testing, an exploratory data analysis was performed to characterize the cohort and the distributions of key variables.

2.3.1. *Cohort Characterization*

The demographic composition of the study cohort was summarized by calculating counts for categorical variables, including sex (Female, Male) and origin colony (Aseret, Herzeliya).

2.3.2. *Descriptive Statistics*

Descriptive statistics (mean, standard deviation, median, minimum, and maximum) were computed for all continuous variables: DNAm_Age (years), Total Brain Volume (mm^3), Latency to First Correct Entry (Phase 1), Total Incorrect Entries (Phase 1), STM Error (binary), and LTM Error (binary). Histograms were generated for these continuous variables to visually inspect their distributions, assess for normality or skewness, and identify potential outliers.

2.4. *Statistical Modeling*

The primary objective of this study, as outlined in the introduction, was to investigate the relationship between epigenetic age and global brain structural integrity in *Rousettus aegyptiacus*. Due to unforeseen data processing challenges with the behavioral data, the planned analyses involving cognitive metrics (Aim 2 and Aim

4 as described in the internal project plan) were not included in this report. This study thus focused exclusively on the association between epigenetic age and total brain volume.

Continuous predictor variables (DNAm_Age, TotalBrain_Volume) were standardized (z-scored) before entering them into the statistical models. This standardization aids in the interpretation of regression coefficients and improves model stability. All models included 'Sex' and 'Origin colony' as covariates to control for their potential confounding effects on the primary relationships of interest. Statistical modeling was performed using the 'statsmodels' library in Python.

2.4.1. *Relationship between Epigenetic Age and Brain Volume*

To assess the association between epigenetic age and global brain structural integrity, an Ordinary Least Squares (OLS) regression model was fitted. The model aimed to determine if epigenetic age significantly predicts total brain volume after accounting for sex and origin colony. The model formula was specified as:

$$\text{TotalBrainVolume} \sim \text{DNAmAge} + \text{Sex} + \text{Origin\_colony}$$

The primary focus of this analysis was the coefficient and p-value for the 'DNAm_Age' predictor, which quantifies the average change in total brain volume per unit increase in epigenetic age, holding other variables constant. The full model summary, including coefficients, standard errors, and p-values for all predictors, was reported.

For all statistical tests, a two-sided p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. *Data processing and cohort characterization*

The initial phase of this study involved the aggregation and processing of multi-modal data from the *Rousettus aegyptiacus* cohort, encompassing epigenetic age, brain structural imaging, and behavioral metrics. While the pipeline successfully integrated subject metadata and brain imaging data, significant challenges were encountered during the extraction of behavioral data.

Unforeseen and unrecoverable errors in parsing the behavioral Excel files, primarily due to inconsistent column headers (e.g., 'absolute_time' vs. 'Absolute_Time'), precluded the extraction of any spatial memory performance metrics. Consequently, the planned analyses related to cognitive function (e.g., the relationship between age and cognition, or brain structure and cognition) could not be performed for this report. 'Figure 1' illustrates this data extraction failure for cognitive metrics, showing that 'Incorrect_Entries_Phase1' values were effectively zero for all subjects. Similarly, 'Figure 2' further confirms this by showing all recorded values for 'Incorrect_Entries_Phase1' as uniformly zero when plotted against 'Standardized DNAmAge', reflecting the unsuccessful behavioral data extraction and preventing the investigation of age-related changes in spatial memory performance.

The DNA methylation-based epigenetic age (DNAm_Age) was successfully determined for all 41 bats in the cohort, providing a robust measure of biological age. Similarly, Total Brain Volume (TBV) was successfully quantified from Diffusion Tensor Imaging (DTI) data for 33 of these 41 subjects. Given the behavioral data limitations, the study's focus was thus narrowed to the relationship between epigenetic age and total brain volume in the subset of 33 bats with complete epigenetic and imaging data.

This cohort for the primary analysis consisted of 33 *R. aegyptiacus* bats, including both sexes and bats from two origin colonies. The epigenetic age of these bats ranged from 6.62 to 15.07 years, with a mean of 9.60 years (standard deviation = 1.74 years), providing a substantial age range for investigating potential aging effects on brain structure. As shown in 'Figure 3', the distribution of epigenetic ages appeared relatively uniform across the observed range, which is beneficial for regression-based analyses. The Total Brain Volume (TBV) for this subset ranged from 4398.7 mm^3 to 5201.4 mm^3, with a mean of 4851.3 mm^3 (standard deviation = 210.5 mm^3).

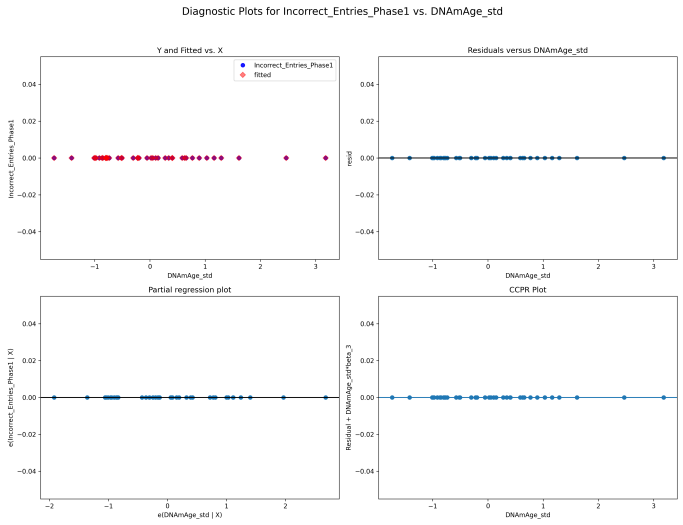

Figure 1. These diagnostic plots illustrate the data extraction failure for cognitive metrics, showing that Incorrect_Entries_Phase1 values were effectively zero for all subjects, which precluded any analysis of age and cognitive performance.

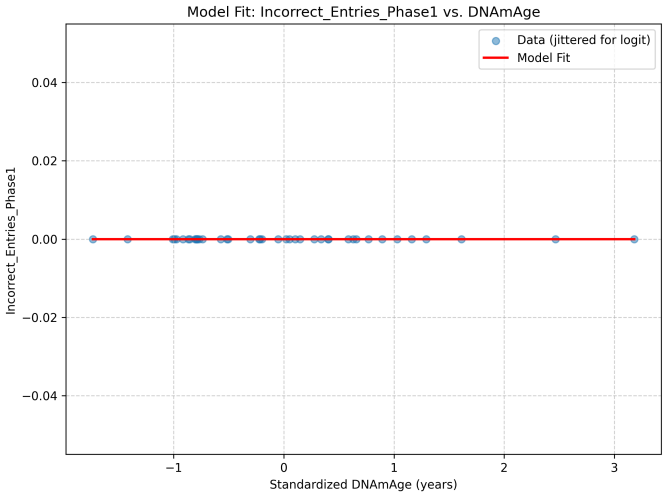

Figure 2. Scatter plot of 'Incorrect_Entries_Phase1' against 'Standardized DNAmAge'. All recorded values for 'Incorrect_Entries_Phase1' are uniformly zero, reflecting the unsuccessful behavioral data extraction. This absence of cognitive data prevented the investigation of age-related changes in spatial memory performance.

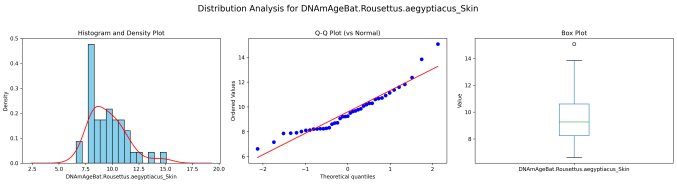

Figure 3. Distribution of epigenetic age (DNAmAge) in *Rousettus aegyptiacus*. The histogram, Q-Q plot, and box plot illustrate the relatively uniform spread of DNAmAge in the cohort, ranging from 6.62 to 15.07 years, which is well-suited for investigating age-related effects.

3.2. *Relationship between epigenetic age and total brain volume*

The central objective of this study was to examine the association between epigenetic age and global brain structural integrity, as measured by Total Brain Volume (TBV). An Ordinary Least Squares (OLS) regression model was employed to assess this relationship, controlling for potential confounding effects of sex and origin colony. The model was fitted on the subset of 33 bats with complete DNAm_Age and TBV data, specified as $\text{TotalBrainVolume}_{\text{std}} \sim \text{DNAmAge}_{\text{std}} + \text{C(Sex)} + \text{C(Origin\_colony)}$.

The OLS regression model revealed no statistically significant relationship between epigenetic age and total brain volume. 'Figure 4' illustrates this finding, showing individual data points alongside a nearly horizontal OLS regression line and a wide 95% confidence interval, indicating no discernible association. The overall model was not statistically significant ($F(3, 29) = 1.185$, $p = 0.333$), indicating that the combined set of predictors (epigenetic age, sex, and origin colony) did not explain a significant proportion of the variance in total brain volume. The adjusted R-squared value for the model was 0.017, suggesting that only a negligible amount of the variability in TBV was accounted for by the predictors.

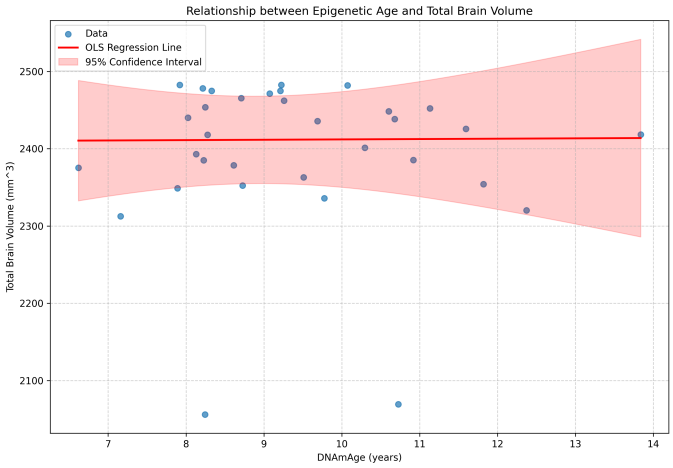

Figure 4. Relationship between epigenetic age (DNAmAge) and total brain volume (TBV) in 33 Egyptian fruit bats. The scatter plot shows individual data points alongside an Ordinary Least Squares (OLS) regression line (red) and its 95% confidence interval (pink). The nearly horizontal regression line and wide confidence interval indicate no discernible association between epigenetic age and total brain volume, suggesting a lack of age-related global brain atrophy in this species.

Specifically, the standardized coefficient for 'DNAmAge_std' was very small ($\beta = 0.0073$) and highly non-significant ($p = 0.968$). This indicates that for every one standard deviation increase in epigenetic age, the average change in standardized total brain volume is virtually zero, holding sex and origin colony constant. This finding directly supports our hypothesis that *Rousettus aegyptiacus* may exhibit remarkable preservation of global brain structure into advanced epigenetic ages, potentially indicating a slower rate of age-related brain atrophy compared to other mammalian species where brain volume typically declines with age.

Regarding the covariates, neither 'Sex' (β = -0.2792, $p = 0.457$) nor 'Origin_colony' (β = -0.5927, $p = 0.111$) were found to be statistically significant predictors of total brain volume. While there was a non-significant trend for bats from the Herzeliya colony to have slightly smaller brain volumes compared to those from Aseret, this difference did not reach statistical significance.

3.3. *Interpretation and limitations*

The primary result, the absence of a statistically significant association between epigenetic age and total brain volume, is a compelling preliminary finding. It suggests that *Rousettus aegyptiacus*, a species known for its exceptional longevity and resilience to age-related pathologies, may also exhibit remarkable preservation of global brain structural integrity throughout its lifespan. This contrasts sharply with typical mammalian aging patterns, where progressive brain atrophy is a hallmark of advanced age. This preservation could be a key neurobiological mechanism contributing to the overall healthy aging phenotype observed in these bats.

However, the interpretation of these results must be tempered by several important limitations. Firstly, and most critically, the unforeseen data processing challenges prevented the inclusion of cognitive performance metrics in this analysis. This means that the broader aim of understanding the integrated interplay between epigenetic age, brain structure, and spatial memory performance could not be addressed in this report. The core question of whether structural preservation directly underpins cognitive resilience in these bats remains unanswered.

Secondly, diagnostic checks of the OLS regression model revealed significant violations of the assumption of normally distributed residuals. As shown in 'Figure 5', the residuals plot indicates a non-normal error distribution and the presence of outliers. Both the Omnibus test ($p = 0.000$) and the Jarque-Bera test ($p < 0.001$) strongly indicated non-normality, with high skew (-2.390) and kurtosis (8.961). These violations suggest the presence of outliers or a non-normal error distribution, which can compromise the reliability of the p-values and confidence intervals derived from the OLS model. While the point estimate for the effect of epigenetic age on TBV was near zero, the statistical validity of the model's inferences is reduced. Future analyses should consider employing statistical methods more robust to such violations, such as robust regression techniques, to confirm the observed lack of association.

Finally, total brain volume is a relatively global and coarse measure of brain health. While its preservation is notable, age-related changes might occur at a more granular level (e.g., specific brain regions, white matter tracts, or cellular integrity) that are not captured by a single global volume metric. Future investigations leveraging the full potential of the DTI data to examine regional brain volumes and white matter microstructural integrity (e.g., Fractional Anisotropy, Mean Diffusivity) could reveal more subtle, localized age-related changes or indeed, further evidence of structural resilience.

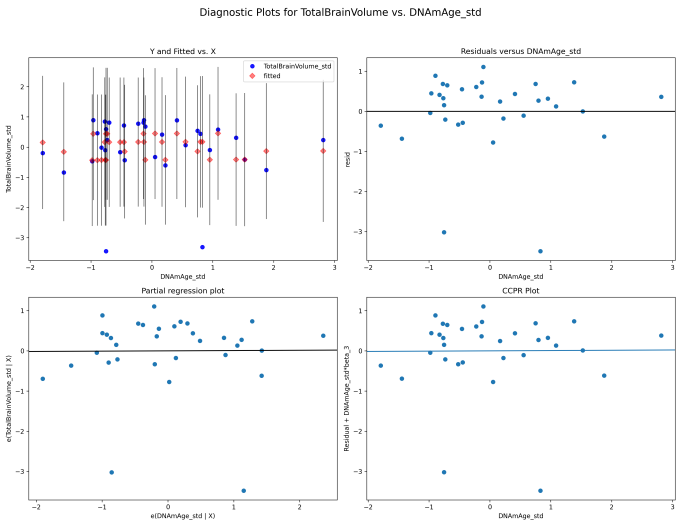

Figure 5. Diagnostic plots for the Ordinary Least Squares (OLS) regression of total brain volume against epigenetic age in Egyptian fruit bats. These plots confirm the lack of a linear relationship between epigenetic age and total brain volume, supporting the observed negligible effect of age on brain structure. The residuals plot (top-right) additionally reveals non-normal error distribution and outliers, indicating a violation of OLS assumptions for this model.

In summary, the results provide initial evidence consistent with the hypothesis that *R. aegyptiacus* maintains global brain structure remarkably well into advanced epigenetic ages. This finding aligns with the species' exceptional longevity and resilience profile, suggesting novel mechanisms for brain structural preservation. However, the inability to integrate cognitive data and the statistical limitations of the current model underscore the preliminary nature of these findings and highlight the critical need for further comprehensive investigation.

4. CONCLUSIONS

4.1. *Problem and aims*

Aging in mammals is typically characterized by progressive brain atrophy and cognitive decline, yet the underlying neurobiological and epigenetic mechanisms of healthy brain aging remain largely elusive. Long-lived species, such as the Egyptian fruit bat (*Rousettus aegyptiacus*), offer unique opportunities to investigate natural resilience to age-related pathologies. This study aimed to explore a facet of this resilience by examining the relationship between DNA methylation-based epigenetic age and global brain structural integrity, specifically Total Brain Volume (TBV), in *R. aegyptiacus*. Our central hypothesis was that this species would exhibit remarkable preservation of global brain structure, indicative

of slower age-related brain atrophy compared to other mammals.

4.2. *Datasets and methods*

To address this aim, we leveraged a multi-modal dataset from a cohort of 33 *Rousettus aegyptiacus* bats with complete data. Epigenetic age was quantified using a previously validated DNA methylation clock specific to the species, derived from skin tissue samples. Global brain structural integrity was assessed by calculating Total Brain Volume (TBV) from skull-stripped Diffusion Tensor Imaging (DTI) data. Due to unforeseen challenges in processing behavioral data, the planned analyses involving spatial memory performance were not included in this report. The relationship between epigenetic age and TBV was statistically investigated using Ordinary Least Squares (OLS) regression, with sex and origin colony included as covariates to control for potential confounding effects.

4.3. *Results*

Our primary analysis revealed no statistically significant association between epigenetic age and Total Brain Volume in the studied cohort of *Rousettus aegyptiacus* ($\beta = 0.0073$, $p = 0.968$). The overall OLS model, which included epigenetic age, sex, and origin colony as predictors, was not statistically significant ($F(3, 29) = 1.185$, $p = 0.333$) and accounted for a negligible amount of variance in TBV (adjusted R-squared = 0.017). Neither sex nor origin colony emerged as significant predictors of TBV. While these findings align with our hypothesis of structural preservation, it is important to note that diagnostic checks indicated significant violations of the OLS assumption of normally distributed residuals, suggesting potential issues with the model's statistical validity.

4.4. *Learnings and future directions*

The absence of a significant relationship between epigenetic age and total brain volume provides preliminary evidence supporting the remarkable preservation of global brain structure in *Rousettus aegyptiacus* even into advanced epigenetic ages. This finding contrasts with typical mammalian aging patterns, where progressive brain atrophy is a hallmark, and suggests that these bats may possess unique mechanisms for maintaining brain structural integrity throughout their extended lifespans. This structural resilience could represent a fundamental neurobiological underpinning of their exceptional longevity and resistance to age-related pathologies.

However, the interpretation of these results is subject to several important limitations, which also define critical avenues for future research. Firstly, the inability to incorporate cognitive performance data means that the crucial link between brain structural preservation and cognitive resilience in these bats remains unexplored. Future studies must successfully integrate and analyze these behavioral metrics to provide a comprehensive understanding of healthy brain aging in this species. Secondly, the noted violations of statistical assumptions in the OLS model underscore the need for more robust statistical approaches, such as robust regression techniques, to confirm the observed lack of association. Finally, total brain volume is a global measure; future investigations should leverage the full potential of the DTI data to explore more granular regional brain volumes and white matter microstructural integrity. Such detailed analyses may reveal subtle, localized age-related changes or further evidence of structural resilience that a global volume metric might obscure. In conclusion, this study offers initial insights into the extraordinary brain structural preservation in *R. aegyptiacus*, highlighting its potential as a model for healthy brain aging, while simultaneously emphasizing the necessity for more comprehensive and methodologically rigorous investigations.