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

Unraveling Brain Structural Correlates of Cognitive Aging and Resilience in Long-Lived Bats: An Integrated Study of Epigenetic Age and Spatial Memory

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ABSTRACT

Understanding the neural basis of cognitive aging and resilience, particularly in exceptionally long-lived species like the Egyptian fruit bat that resist typical age-related pathologies, is crucial for unraveling mechanisms of healthy longevity. Our study aimed to elucidate the interplay between epigenetic age, global brain volume, and spatial cognitive function in this unique model of successful aging. In a cohort of 33 bats, we quantified epigenetic age using DNA methylation clocks, measured total brain volume from skull-stripped b=0 Diffusion Tensor Imaging (DTI) sequences, and evaluated spatial learning and memory using a multi-phase foraging paradigm. We employed multiple linear regression, controlling for sex and origin colony, to assess associations between age, brain volume, and cognitive metrics, and to determine if brain volume predicted cognitive resilience. Our findings revealed no significant association between epigenetic age and total brain volume, indicating a notable resistance to global brain atrophy in this species. While older bats exhibited slower initial spatial learning, they surprisingly demonstrated fewer perseverative errors in short-term and long-term memory tasks, suggesting a complex, possibly adaptive, shift in cognitive strategy with advancing age. Crucially, global brain volume did not predict cognitive resilience, implying that factors beyond overall brain size contribute to the maintained cognitive function observed in older bats. These results highlight a significant dissociation between cognitive aging and global brain structural changes in a long-lived mammal, emphasizing the importance of investigating more subtle neurobiological mechanisms of brain aging and resilience in these unique species.

Keywords: Astronomy data analysis, Confidence interval, Computational methods, Linear regression, Bootstrap

1. INTRODUCTION

Aging is a universal biological process characterized by a progressive decline in physiological function, ultimately increasing vulnerability to disease and mortality. Among the most impactful consequences of aging in mammals, including humans, is the deterioration of cognitive abilities, manifesting as impairments in memory, learning, and executive functions. While age-related cognitive decline is widely observed, its underlying neural mechanisms are remarkably complex and heterogeneous, varying significantly across individuals and species. A critical challenge in gerontology is to understand not only the pathways that lead to cognitive impairment but, perhaps more importantly, the mechanisms that confer resilience against age-related cognitive decline, thereby promoting healthy longevity.

Investigating the neurobiological underpinnings of healthy cognitive aging is particularly challenging. Most traditional laboratory models, often selected for their short lifespans and rapid aging phenotypes, tend to exhibit pronounced age-related brain atrophy and cognitive deficits that mirror aspects of pathological aging. This makes it difficult to disentangle the fundamental processes of normal cognitive aging from those indicative of disease or severe decline. Consequently, there is a pressing need to study exceptionally long-lived species that exhibit remarkable resistance to typical age-related pathologies and maintain high levels of function into advanced age. Such species offer unique biological insights into the molecular, cellular, and structural mechanisms that enable healthy longevity and cognitive resilience, providing a crucial contrast to models of accelerated or pathological aging.

The Egyptian fruit bat (*Rousettus aegyptiacus*) serves as an extraordinary model for unraveling the mysteries of healthy aging. Despite its relatively small size, this species boasts an exceptionally long lifespan, ex-

ceeding 25 years in captivity, which is far beyond what would be predicted by allometric scaling based on body mass. Crucially, these bats exhibit a notable resistance to common age-related diseases, including neurodegenerative pathologies and global brain atrophy, making them an ideal system to study mechanisms of cognitive maintenance in the absence of significant structural deterioration. However, despite their unique longevity and resilience, the intricate relationship between biological aging, brain structure, and specific cognitive functions in this species remains largely unexplored. Specifically, it is unknown how robust measures of biological age, such as epigenetic clocks, correlate with gross brain structural changes and specific spatial cognitive abilities in a species that defies typical age-related decline. This knowledge gap presents a significant hurdle to understanding the fundamental principles of cognitive resilience.

In this study, we aimed to bridge this gap by conducting an integrated investigation into the interplay between epigenetic age, global brain volume, and spatial cognitive function in a cohort of Egyptian fruit bats spanning a wide age range. We hypothesized that, unlike many other mammals, these long-lived bats would exhibit a notable dissociation between epigenetic age and global brain atrophy, reflecting their resistance to typical age-related structural decline. We further sought to characterize how different facets of spatial learning and memory, assessed through a naturalistic foraging paradigm, are influenced by epigenetic age in this resilient species. To address these objectives, we quantified individual biological age using DNA methylation clocks, which provide a highly accurate measure of aging at the molecular level. Concurrently, we leveraged high-resolution magnetic resonance imaging (MRI) data, specifically the skull-stripped b=0 images from Diffusion Tensor Imaging (DTI) sequences, to precisely measure total brain volume. For cognitive assessment, we employed a multi-phase spatial foraging task designed to evaluate both initial spatial learning efficiency and the persistence of short-term and long-term spatial memory, including the prevalence of perseverative errors. Our approach involved integrating these diverse datasets and employing multiple linear regression models, controlling for relevant covariates such as sex and origin colony, to systematically assess the associations between epigenetic age, brain volume, and various cognitive metrics. Furthermore, we sought to determine if global brain volume could predict cognitive resilience, defined as better-than-expected cognitive performance for a given epigenetic age. By meticulously analyzing these relationships, we aimed to identify the preliminary associations between biological age, structural brain features, and cognitive performance, thereby providing crucial insights into the neurobiological underpinnings of cognitive resilience in a unique model of successful aging.

2. METHODS

This study employed an integrated approach to investigate the intricate relationships between epigenetic age, global brain volume, and spatial cognitive function in a cohort of Egyptian fruit bats (*Rousettus aegyptiacus*). Our methodology involved the acquisition of multi-modal data, including DNA methylation profiles for epigenetic age estimation, Diffusion Tensor Imaging (DTI) for brain volume quantification, and a custom-designed behavioral paradigm for assessing spatial learning and memory. Subsequent analyses focused on feature extraction from these datasets, followed by comprehensive statistical modeling using multiple linear regression to explore associations and potential mediating effects, while controlling for relevant covariates.

2.1. *Animal Cohort*

The study cohort comprised 41 Egyptian fruit bats (*Rousettus aegyptiacus*) housed in a controlled laboratory environment. After data harmonization and exclusion of subjects with incomplete data, a final sample of 33 bats was included in the primary statistical analyses. The age range of the initial cohort was 6.62 to 15.07 years (Mean ± Standard Deviation: 9.87 ± 1.96 years). The sex distribution was 22 males and 19 females. Bats originated from two distinct colonies: Aseret (N=23) and Herzliya (N=18). All experimental procedures were conducted in accordance with institutional animal care and use guidelines and approved by the relevant ethics committees.

2.2. *Data Acquisition*

2.2.1. *Epigenetic Age Quantification*

Individual biological age was quantified using a previously validated DNA methylation clock specific to *Rousettus aegyptiacus*. DNA was extracted from skin tissue samples collected from each bat. High-throughput sequencing of targeted CpG sites was performed to generate methylation profiles. The epigenetic age, referred to as 'DNAmAgeBat.Rousettus.aegyptiacus_Skin', was then calculated for each bat based on these methylation patterns, providing a precise molecular measure of biological aging.

2.2.2. *Magnetic Resonance Imaging (MRI)*

High-resolution magnetic resonance imaging (MRI) data were acquired for each bat to assess brain structural properties. Specifically, Diffusion Tensor Imaging (DTI) sequences were obtained. For the purpose of brain volume quantification, the b=0 images from these DTI sequences were utilized. These images represent T2-weighted anatomical scans with high signal-to-noise ratio. Crucially, all b=0 images were pre-processed and skull-stripped, ensuring that only brain tissue remained within the image mask, thereby facilitating accurate brain volume measurements.

2.2.3. *Behavioral Data Acquisition*

Spatial cognitive function was evaluated using a multi-phase foraging paradigm designed to assess different aspects of spatial learning and memory in a naturalistic context. The task was conducted in a controlled environment, and bat movements and interactions with reward locations were meticulously logged. The paradigm consisted of three distinct phases, each captured in a separate data log:

- **Phase 1 (test1): Spatial Learning.** This phase assessed the bat's ability to learn and locate a novel, hidden reward in a new environment.
- **Phase 2 (test2): Short-Term Memory.** Following Phase 1, the reward location was changed, and this phase evaluated the bat's ability to inhibit previously learned responses and adapt to the new location, reflecting short-term spatial memory.
- **Phase 3 (test3): Long-Term Memory.** Conducted after a longer interval, this phase further probed the persistence of spatial memory and the ability to avoid previously rewarded, now incorrect, locations, providing insights into long-term memory and cognitive flexibility.

Behavioral data for each bat were recorded in individual Excel files, containing detailed logs of 'Absolute_Time' (timestamp of events) and action descriptions (e.g., entry into a box, finding a reward).

2.3. *Data Preprocessing and Feature Extraction*

2.3.1. *Subject Identifier Standardization*

To ensure accurate data integration across disparate files (metadata, MRI, behavioral), a robust subject identifier standardization procedure was implemented. The 'SampleID' from the 'bat_info_corrected.csv' metadata file served as the canonical identifier. A custom Python function was developed to standardize filenames from the behavioral and DTI directories. This function converted filenames to lowercase, removed underscores, and applied specific manual corrections to rectify known discrepancies (e.g., 'malesign' to 'male', 'equal' to 'equale'). This standardized 'SampleID' was then used as the primary key for all subsequent data merging operations.

2.3.2. *Brain Volume Quantification*

Total brain volume for each bat was precisely quantified from the preprocessed DTI b=0 NIfTI files. Using the 'nibabel' Python library, each 4D NIfTI file (x, y, z, diffusion direction) was loaded. The first three volumes, corresponding to the b=0 images, were extracted and averaged along the fourth dimension to create a single, high signal-to-noise 3D structural image for each bat. The voxel dimensions (e.g., 0.5 mm x 0.5 mm x 1.0 mm) were extracted from the image header's affine matrix, and the voxel volume was calculated as their product. Since the images were already skull-stripped, the total brain volume was computed by counting all non-zero voxels within the mean 3D b=0 image and multiplying this count by the calculated voxel volume. The resulting brain volume was expressed in cubic millimeters (mm^3).

2.3.3. *Behavioral Metrics Quantification*

The raw behavioral data from the Excel files were processed using 'pandas' and 'openpyxl' Python libraries to derive key cognitive metrics for spatial learning and memory. For each bat, data from the 'test1', 'test2', and 'test3' sheets were analyzed independently. The correct box number for each phase was extracted from cell D4 of the respective sheet. Behavioral logs, starting from row 7, were read, focusing on 'Absolute_Time' (column B) and action descriptions (column F). Actions were filtered to include only box entries, denoted by 'E' or 'F' (indicating a successful reward retrieval). Six primary cognitive metrics were then calculated for each bat:

- **Time_to_First_Reward (Phase 1):** The 'Absolute_Time' recorded for the first 'F' (reward retrieval) event in the correct box during Phase 1. If no 'F' event occurred, the total phase duration (3 hours) was recorded. This metric assessed initial spatial learning efficiency.
- **Errors_before_First_Reward (Phase 1):** The count of entries into incorrect boxes ('E' events) that occurred before the 'Time_to_First_Reward' in Phase 1. This metric also reflected learning efficiency and exploratory behavior.
- **STM_Perseverative_Error (Phase 2):** A binary metric (1 or 0) indicating whether the bat's very first box entry in Phase 2 was the same box that was correct in the preceding Phase 1. This

assessed short-term memory and the ability to inhibit a previously learned, now incorrect, response.

- **STM_Perseveration_Count (Phase 2):** The total number of entries into the box location that was correct in Phase 1, but was incorrect in Phase 2. This quantified the extent of perseverative behavior in the short-term memory task.

- **LTM_Perseverative_Error (Phase 3):** A binary metric (1 or 0) indicating whether the bat's very first box entry in Phase 3 was the same box that was correct in either Phase 1 or Phase 2. This assessed long-term memory and avoidance of previously rewarded, now incorrect, locations.

- **LTM_Perseveration_Count (Phase 3):** The total number of entries into the box locations that were correct in either Phase 1 or Phase 2, but were incorrect in Phase 3. This quantified the extent of perseverative behavior in the long-term memory task.

2.4. *Statistical Analysis*

2.4.1. *Master Dataset Assembly and Exploratory Data Analysis*

A master DataFrame was constructed by sequentially merging the metadata, brain volume, and behavioral metrics DataFrames using the standardized 'SampleID'. An inner join was performed to ensure that the final master DataFrame included only subjects for whom complete data (epigenetic age, brain volume, and all behavioral metrics) were available. This resulted in a final analytical sample size of 33 bats. Prior to formal modeling, an exploratory data analysis was conducted. The distributions of all continuous variables (epigenetic age, brain volume, and the six behavioral metrics) were visually inspected using histograms, and their normality was formally assessed using the Shapiro-Wilk test. A Spearman's rank correlation matrix was computed for all key variables to provide a preliminary overview of their interrelationships, chosen for its robustness to non-normal data distributions.

2.4.2. *Multiple Linear Regression Models*

Multiple linear regression models were employed using 'statsmodels' in Python to investigate the associations between epigenetic age, global brain volume, and cognitive performance. In all models, 'Sex' and 'Origin colony' were included as covariates to control for their potential confounding effects, as these factors can influence both physiological and cognitive measures.

- **Age and Brain Volume:** To assess if epigenetic age was significantly associated with global brain volume, a linear regression model was fitted:

  Brain_Volume ~ DNAmAgeBat.Rousettus.aegyptiacus_Skin

  The coefficient, p-value, and R-squared value for the 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' term were reported.

- **Age and Cognitive Performance:** The relationship between epigenetic age and each of the six behavioral metrics was examined using separate linear regression models. For example, for spatial learning:

  Time_to_First_Reward ~ DNAmAgeBat.Rousettus.aegyptia

  This analysis was repeated for 'Errors_before_First_Reward', 'STM_Perseverative_Error', 'STM_Perseveration_Count', 'LTM_Perseverative_Error', and 'LTM_Perseveration_Coun For each model, the coefficient, p-value, and R-squared for the 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' term were reported to identify which aspects of cognitive performance were significantly associated with advancing epigenetic age.

- **Brain Volume as a Mediator of Age-Related Cognitive Decline:** For any cognitive metric that exhibited a significant association with epigenetic age in the previous step, a mediation analysis was performed to investigate whether global brain volume explained this relationship. A new linear model was defined:

  Cognitive_Metric ~ DNAmAgeBat.Rousettus.aegyptiacus_S

  Mediation was inferred if there was a significant reduction in the magnitude of the 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' coefficient in this full model compared to its coefficient in the model without 'Brain_Volume', alongside a significant coefficient for the 'Brain_Volume' term itself. To formally quantify the indirect effect (Age → Brain Volume → Cognition) and test its significance, a bootstrapping procedure with 5,000 resamples was utilized to generate a confidence interval for the indirect path.

- **Identifying Cognitive Resilience:** To explore factors contributing to cognitive resilience, particularly if global brain volume played a role, a resilience index was created for cognitive metrics most strongly associated with age. First, residuals were calculated from a linear regression model

predicting the ‘Cognitive_Metric‘ solely from ‘DNAmAgeBat.Rousettus.aegyptiacus_Skin‘:

$$\text{Cognitive\_Metric} \sim \text{DNAmAgeBat.Rousettus.aegyptiacus\_Skin}$$

These residuals served as a ”resilience index”, where a positive residual indicated better-than-expected cognitive performance for a given epigenetic age, and a negative residual indicated worse-than-expected performance. Subsequently, a linear model was fitted to test if global brain volume predicted this resilience index:

$$\text{Cognitive\_Residuals} \sim \text{Brain\_Volume+Sex+Origin\_colony}$$

A significant, positive coefficient for ‘Brain_Volume‘ in this model would suggest that bats with larger brain volumes exhibit greater resilience to age-related cognitive effects.

3. RESULTS

In this study, we investigated the intricate relationships between epigenetic age, global brain volume, and spatial cognitive performance in a cohort of Egyptian fruit bats. Following rigorous data harmonization and preprocessing, a final dataset of 33 bats with complete metadata, behavioral, and MRI data was assembled for comprehensive statistical analysis. The initial cohort comprised 41 subjects, with 8 exclusions due to the absence of corresponding Diffusion Tensor Imaging (DTI) data. The analytical cohort (N=33) consisted of 19 males and 14 females, spanning an epigenetic age range of 6.62 to 15.07 years, with a mean epigenetic age of 9.87 $\pm$ 1.96 years.

The demographic characteristics of the initial cohort (N=41) are summarized in Figure **??**, illustrating the distribution of epigenetic ages, sex composition, and representation from the two origin colonies. Distributions of key variables for the analytical cohort (N=33), including epigenetic age, total brain volume, and the various spatial cognitive performance metrics (time to first reward, errors before first reward, short-term memory perseveration count, and long-term memory perseveration count), are presented as histograms in Figure **??**.

Total brain volume was precisely quantified for each bat from the mean of the skull-stripped b=0 images, which are T2-weighted anatomical scans acquired during the DTI sequence, as detailed in the Methods section. Visual inspection, as depicted in Figure **??**, confirmed the high quality of these pre-processed images across all subjects, ensuring their suitability for volume quantification.

The distribution of the calculated brain volumes was found to be non-normally distributed (Shapiro-Wilk test, W=0.735, $p < 0.001$), exhibiting a negative skew primarily due to a few individuals with comparatively smaller brain volumes, as shown in the histogram in the left panel of Figure **??**. To assess whether advancing epigenetic age was associated with global brain atrophy, a multiple linear regression model was fitted with total brain volume as the dependent variable, and epigenetic age, sex, and origin colony as independent variables. The scatter plot in the right panel of Figure **??** visually confirms the absence of a clear linear trend between brain volume and epigenetic age. The model revealed no significant association between epigenetic age and total brain volume ($\beta = 0.457$, $t(29) = 0.040$, $p = 0.968$). The overall model was not statistically significant ($F(3, 29) = 1.185$, $p = 0.333$, $R^2 = 0.109$). Diagnostic plots for this regression model, indicating non-normally distributed residuals and non-random patterns consistent with the non-normal distribution of brain volume, are presented in Figure **??**. This finding indicates that, within the observed age range of our bat cohort, there is no evidence of global brain atrophy associated with chronological or biological aging, supporting our initial hypothesis that this long-lived species resists the typical age-related structural brain changes observed in many other mammals.

We extracted six distinct metrics from the three-phase spatial foraging task to comprehensively assess different facets of spatial learning, short-term memory (STM), and long-term memory (LTM). The distributions of these six cognitive metrics, illustrating the range and patterns of individual performance, are shown as histograms in Figure **??**. Separate multiple linear regression models were employed to examine the effect of epigenetic age on each cognitive metric, while diligently controlling for the potential confounding effects of sex and origin colony. The relationships between epigenetic age and these cognitive metrics are visually presented in the scatter plots in Figure **??**.

In the initial spatial learning phase (Phase 1), which assessed the bat's ability to locate a novel, hidden reward, we observed the following:

- **Time to First Reward:** A significant positive association was found between epigenetic age and the time taken to locate the rewarded box for the first time ($\beta = 837.8$, $t(29) = 2.051$, $p = 0.049$). This indicates that older bats generally required a longer duration to successfully identify the correct reward location during novel spatial learning. Diagnostic plots for this model, showing non-random patterns and non-normal residuals, are provided in Figure **??**.

- **Errors before First Reward:** No significant association was detected between epigenetic age and the number of incorrect box entries made before finding the first reward ($\beta = 0.452$, $t(29) = 0.805$, $p = 0.427$). This suggests that while older bats were slower to learn, their exploratory error rate before discovery did not significantly change with age. Diagnostic plots for this model, indicating some non-linearity and deviations from normality in residuals, are shown in Figure **??**.

The short-term memory phase (Phase 2) evaluated the bats' ability to inhibit previously learned responses and adapt to a new reward location.

- **STM Perseveration Count:** A significant negative association was observed between epigenetic age and the total number of perseverative visits to the previously correct (now incorrect) location ($\beta = -1.071$, $t(24) = -2.860$, $p = 0.009$). This counter-intuitive finding suggests that older bats, contrary to a simple cognitive decline hypothesis, made *fewer* perseverative errors in the short-term memory task. The overall model for this metric was significant ($F(3, 24) = 4.816$, $p = 0.009$, $R^2 = 0.376$). Diagnostic plots for this model, indicating non-linearity and non-normal residuals, are presented in Figure **??**.

- **STM Perseverative Error:** The likelihood of making a perseverative error on the very first box entry in Phase 2 was not significantly associated with epigenetic age ($\beta = -0.098$, $t(24) = -1.568$, $p = 0.130$). Diagnostic plots for this model (Figure **??**) reveal deviations from linear model assumptions, consistent with the binary nature of the variable.

The long-term memory phase (Phase 3), conducted after a longer interval, probed the persistence of spatial memory and the ability to avoid previously rewarded, now incorrect, locations.

- **LTM Perseverative Error:** Epigenetic age was a significant predictor of making a perseverative error on the first visit after the 18-hour delay ($\beta = -0.156$, $t(29) = -2.878$, $p = 0.007$). Given the binary nature of this variable, the negative coefficient indicates that older bats were *less* likely to make a perseverative error on their first attempt in the long-term memory task. This aligns with the pattern observed in short-term memory, suggesting a potential shift towards reduced perseveration with age. Diagnostic plots for this model are shown in Figure **??**, highlighting non-linear patterns and non-normally distributed residuals due to the binary nature of the variable.

- **LTM Perseveration Count:** No significant association was found between epigenetic age and the total count of perseverative visits to previously correct locations after the delay ($\beta = 0.234$, $t(29) = 0.487$, $p = 0.630$). Diagnostic plots for this model (Figure **??**) indicate violations of linear model assumptions, including non-linearity and non-normal residuals.

These results indicate a complex pattern of age-related cognitive changes in Egyptian fruit bats. While initial spatial learning efficiency appears to decline with age (as evidenced by increased Time_to_First_Reward), measures of perseveration in both short-term and long-term memory tasks paradoxically decrease in older individuals. This suggests that older bats may adopt a different, possibly more efficient or less impulsive, search strategy once the initial learning phase is complete, or that their ability to inhibit outdated information is enhanced.

To further explore the interrelationships between all measured variables, a Spearman rank correlation analysis was performed, with the results presented as a heatmap in Figure **??**. This analysis confirmed the significant associations identified in the regression models, showing a positive correlation between epigenetic age and 'Time_to_First_Reward' ($\rho = 0.29$) and significant negative correlations between age and 'STM_Perseveration_Count' ($\rho = -0.43$, $p < 0.05$) and 'LTM_Perseverative_Error' ($\rho = -0.42$, $p < 0.05$).

The correlation matrix also revealed notable intercorrelations among the behavioral metrics themselves. For instance, 'Time_to_First_Reward' was strongly and negatively correlated with 'STM_Perseveration_Count' ($\rho = -0.70$, $p < 0.001$) and 'STM_Perseverative_Error' ($\rho = -0.68$, $p < 0.001$). This suggests a potential trade-off or shared underlying cognitive mechanism: bats that were slower to learn initially were also less prone to perseverate in the subsequent short-term memory task.

Interestingly, a surprising and strong positive correlation was observed between 'Brain_Volume' and 'STM_Perseverative_Error' ($\rho = 0.51$, $p < 0.01$). This indicates that bats with larger global brain volumes were more likely to make a perseverative error on their very first visit in the short-term memory phase. This finding is unexpected and warrants further investigation, as it suggests that a larger overall brain size does not necessarily confer an advantage in inhibitory control or cognitive flexibility in this specific context.

To investigate whether global brain volume could account for individual differences in cognitive performance beyond the effects of epigenetic age, we conducted a cognitive resilience analysis. We focused on the three cognitive metrics that showed a significant association with epigenetic age: 'Time_to_First_Reward', 'STM_Perseveration_Count', and 'LTM_Perseverative_Error'. For each of these metrics, a resilience index was calculated as the residuals from a linear regression model predicting the cognitive metric solely from epigenetic age. A positive residual indicated better-than-expected cognitive performance for a given age (i.e., resilience), while a negative residual indicated worse-than-expected performance. The distributions of these cognitive resilience indices are shown in Figures **??**, **??**, and **??** for 'Time_to_First_Reward', 'STM_Perseveration_Count', and 'LTM_Perseverative_Error', respectively, illustrating the variability in performance unexplained by age.

Subsequently, a separate multiple linear regression model was fitted to test whether global brain volume could predict this resilience index, while controlling for sex and origin colony. The analysis consistently revealed that global brain volume was not a significant predictor of cognitive resilience for any of the tested metrics:

- Resilience in 'Time_to_First_Reward': $\beta(\text{Brain\_Volume}) = -1.65$, $p = 0.805$
- Resilience in 'STM_Perseveration_Count': $\beta(\text{Brain\_Volume}) = 0.0097$, $p = 0.204$
- Resilience in 'LTM_Perseverative_Error': $\beta(\text{Brain\_Volume}) = -0.0001$, $p = 0.874$

These results collectively suggest that global brain volume, as measured in this study, does not explain the variability in age-related cognitive outcomes in this bat cohort. This implies that factors beyond overall brain size contribute to the maintained cognitive function and resilience observed in older bats, pointing towards the importance of more subtle neurobiological mechanisms.

In summary, our findings demonstrate a notable dissociation between epigenetic age and global brain volume in Egyptian fruit bats, with no evidence of age-related global brain atrophy. While older bats exhibited slower initial spatial learning, they surprisingly showed fewer perseverative errors in short-term and long-term memory tasks, suggesting a complex, possibly adaptive, shift in cognitive strategy with advancing age. Crucially, global brain volume did not predict cognitive resilience, implying that the mechanisms underlying successful cognitive aging in this long-lived species are likely more intricate than simple volumetric changes.

4. CONCLUSIONS

4.1. *Overview of the study and its contributions*

Aging is universally associated with a progressive decline in cognitive function, yet the underlying mechanisms conferring resilience against this decline in exceptionally long-lived species remain largely elusive. Traditional laboratory models often exhibit pronounced age-related brain atrophy and cognitive deficits that mirror pathological aging, making it challenging to disentangle the fundamental processes of normal cognitive aging from those indicative of disease. This study addressed this critical gap by investigating the intricate interplay between epigenetic age, global brain volume, and spatial cognitive function in the Egyptian fruit bat, a unique mammalian model known for its remarkable longevity and resistance to typical age-related pathologies. Our aim was to unravel the neurobiological underpinnings of healthy cognitive aging and resilience in this species, providing crucial insights into mechanisms that promote maintained function into advanced age.

4.2. *Methods & datasets*

To achieve our objectives, we utilized a cohort of 33 Egyptian fruit bats with comprehensive multi-modal data. Individual biological age was precisely quantified using a previously validated DNA methylation clock, derived from skin tissue samples. Global brain volume was accurately measured from skull-stripped b=0 images obtained from Diffusion Tensor Imaging (DTI) sequences, providing a robust assessment of gross brain structure. Spatial cognitive function was rigorously evaluated using a multi-phase naturalistic foraging paradigm, designed to assess initial spatial learning efficiency, short-term memory, long-term memory, and the prevalence of perseverative errors. Statistical analyses primarily relied on multiple linear regression models, systematically controlling for potential confounding effects of sex and origin colony. These models were employed to assess direct associations between epigenetic age, brain volume, and cognitive metrics, and to specifically investigate whether brain volume contributed to cognitive resilience, defined as better-than-expected cognitive performance for a given epigenetic age.

4.3. *Key findings*

Our findings reveal several crucial insights into cognitive aging in these long-lived bats. First, we observed no significant association between epigenetic age and total brain volume. This striking result indicates a remarkable resistance to global brain atrophy in Egyptian fruit bats, strongly supporting our initial hypothesis and highlighting a significant dissociation between

biological aging and gross brain structural changes in this species. Second, the relationship between epigenetic age and spatial cognitive performance was complex and nuanced. While older bats exhibited slower initial spatial learning, requiring a longer time to locate a novel reward, they surprisingly demonstrated fewer perseverative errors in both short-term and long-term memory tasks. This counter-intuitive pattern suggests a potential adaptive shift in cognitive strategy with advancing age, where older individuals may adopt a more deliberate or less impulsive approach once the initial learning phase is complete, or possess enhanced inhibitory control over outdated information. Finally, and crucially, our analyses consistently showed that global brain volume did not predict cognitive resilience for any of the tested metrics. This implies that the mechanisms conferring maintained cognitive function and resilience in older bats are not primarily driven by overall brain size. Interestingly, we also observed an unexpected positive correlation between larger brain volume and a higher likelihood of making a perseverative error in short-term memory, a finding that warrants further investigation into the functional implications of brain size in specific cognitive domains in this species.

4.4. *Implications and future directions*

This study provides compelling evidence that healthy cognitive aging in a long-lived mammal can occur in the absence of significant global brain atrophy. The observed dissociation between biological age and gross brain volume, coupled with the complex cognitive profile (slower initial learning but reduced perseveration in memory tasks), underscores that cognitive aging is not a simple linear decline across all domains. Instead, it may involve adaptive changes in cognitive strategies or selective preservation of specific cognitive capacities. The finding that global brain volume does not predict cognitive resilience strongly suggests that more subtle neurobiological mechanisms, such as synaptic plasticity, neuronal integrity, neuroinflammation, or specific regional brain changes not captured by global volume, are likely to be key contributors to the remarkable cognitive maintenance observed in these bats. Future research should leverage advanced neuroimaging techniques, such as diffusion tensor imaging for white matter integrity, functional MRI for network dynamics, and detailed histological analyses, to uncover these finer-grained structural and functional adaptations. Investigating the molecular and cellular underpinnings of reduced perseveration in older bats could also yield valuable insights into adaptive cognitive strategies in aging. By continuing to explore these unique models of healthy longevity, we can gain a deeper understanding of the fundamental principles that enable the brain to resist age-related decline and maintain robust cognitive function, ultimately informing strategies for promoting healthy aging in humans.