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Investigating Cognitive Resilience in Long-Lived Bats: Challenges in Integrating Epigenetic Age, Spatial Memory, and Brain Structure

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

ABSTRACT

Understanding the neural underpinnings of cognitive resilience in exceptionally long-lived species is crucial for uncovering strategies for healthy brain aging. This study aimed to investigate these mechanisms in 41 Egyptian fruit bats (*Rousettus aegyptiacus*) by integrating epigenetic age (DNA methylation age), detailed spatial cognitive performance from a multi-phase foraging paradigm, and brain structural measures derived from MRI, such as whole-brain volume and fractional anisotropy. The original goal was to identify how individual differences in brain structure correlated with biological age and variations in spatial learning, memory, and cognitive flexibility, particularly exploring age-by-brain structure interaction effects. However, the comprehensive analysis was significantly constrained by unforeseen data processing challenges: a critical failure in MRI data processing prevented the extraction of all brain structural measures, and systematic issues during behavioral data parsing limited quantifiable cognitive metrics to only initial learning speed (Time_to_First_Food) and cognitive flexibility (Switch_Cost). From the successfully quantified data, no significant relationship was observed between epigenetic age and either initial spatial learning efficiency or cognitive flexibility. Interestingly, the bats' origin colony significantly predicted cognitive flexibility, suggesting that environmental or genetic factors may exert a stronger influence than epigenetic age on this cognitive domain in this cohort. This research underscores the critical importance of robust data validation pipelines in complex multimodal studies and highlights the persistent technical hurdles in unraveling the intricate interplay of aging, cognition, and brain structure in unique mammalian models.

Keywords: Computational methods, Linear regression, Astronomy software, Computational astronomy, Astrostatistics

1. INTRODUCTION

Healthy brain aging, characterized by the maintenance of cognitive function and the absence of neurodegenerative disease, represents a fundamental challenge in biomedical research. While chronological age is a primary risk factor for cognitive decline, there is significant inter-individual variability in cognitive trajectories across the lifespan. Understanding the biological mechanisms that confer cognitive resilience—the capacity to maintain robust cognitive function despite advancing age or neuropathological burden—is crucial for developing effective strategies to promote brain health in an aging global population.

Long-lived mammalian species offer unique opportunities to investigate the biological underpinnings of exceptional longevity and age-related resilience. Among these, bats (Order Chiroptera) are particularly remarkable, exhibiting lifespans far exceeding predictions based on their body size, often without overt signs of age-related diseases or cognitive decline observed in other mammals. The Egyptian fruit bat, *Rousettus aegyptiacus*, with a maximum recorded lifespan of over 25 years in captivity, represents an ideal model to explore the neural correlates of cognitive resilience. By studying these animals, we can move beyond typical mammalian models and potentially uncover novel, evolutionarily conserved strategies for healthy brain aging.

The core problem addressed by this study is to identify the specific neural attributes that contribute to sustained cognitive performance as biological age advances in these exceptionally long-lived bats, and how these attributes interact with the aging process. This endeavor is inherently difficult due to several factors. Firstly, relying solely on chronological age may not accurately reflect an individual's true biological aging status; more precise molecular markers, such as epigenetic age derived from DNA methylation patterns, are required. Sec-

ondly, assessing complex cognitive domains like spatial learning, memory retention, and cognitive flexibility demands sophisticated, multi-phase behavioral paradigms capable of capturing nuanced individual performance differences. Thirdly, detailed structural characterization of the bat brain necessitates advanced neuroimaging techniques, specifically magnetic resonance imaging (MRI), to derive measures like whole-brain volume and diffusion tensor imaging (DTI) metrics such as fractional anisotropy and mean diffusivity. The paramount difficulty, however, lies in the intricate process of integrating these disparate, high-dimensional datasets—from epigenetic markers of age to nuanced behavioral performance and high-resolution brain imaging—into a coherent analytical framework. Such complex multimodal integration is essential for a comprehensive understanding but is technically challenging and prone to unforeseen hurdles.

To address this challenge, this study aimed to provide an integrated understanding of cognitive resilience in the Egyptian fruit bat. We attempted to solve this problem by comprehensively combining three critical dimensions of biological inquiry: a precise measure of biological age using DNA methylation (epigenetic age), a detailed assessment of spatial memory and cognitive flexibility through a multi-phase foraging paradigm, and detailed brain structural measures derived from MRI. Our core objective was to identify how individual differences in brain structure—such as overall brain volume or white matter integrity indicated by fractional anisotropy—correlate with both epigenetic age and individual variations in spatial learning, memory retention, and cognitive flexibility within our bat cohort. Specifically, we sought to uncover structural signatures associated with maintained cognitive function even in the presence of advanced epigenetic age, through the examination of age-by-brain structure interaction effects. This approach was designed to directly test the hypothesis that specific brain structural features modulate the relationship between biological age and cognitive performance, thereby providing direct evidence for their role in cognitive resilience.

We verified our approach through a systematic, multi-stage analytical pipeline. This involved rigorous data consolidation and harmonization across disparate data types, followed by the quantitative extraction of precise behavioral metrics from extensive raw logs and the derivation of structural and diffusion metrics from raw MRI data. The culmination of this process was a comprehensive statistical analysis. We employed correlational analyses to establish initial relationships between our key variables and, crucially, utilized multiple linear regression models with interaction terms. The general form of these models was:

$$\text{Cognitive Metric} \sim \text{DNAmAge} + \text{Brain Metric} + \text{DNAmAge} \times \text{Brain M}$$

This analytical strategy was specifically designed to directly test the hypothesis that specific brain structural features modulate the relationship between biological age and cognitive performance, thereby providing direct evidence for their role in cognitive resilience. By systematically integrating and analyzing these multimodal data, this research sought to provide novel insights into the biological strategies that underpin healthy brain aging in an exceptionally long-lived mammalian model.

2. METHODS

The methodological approach for this study was designed to integrate three distinct but complementary datasets: precise measures of biological age using DNA methylation, comprehensive behavioral assessments of spatial cognition, and detailed brain structural and diffusion tensor imaging (DTI) metrics. This multi-modal strategy aimed to identify neural attributes contributing to cognitive resilience in Egyptian fruit bats (*Rousettus aegyptiacus*), as outlined in the study's introduction. All data processing and analysis steps were performed using Python, leveraging libraries such as `pandas`, `openpyxl`, `nibabel`, `dipy`, and `statsmodels`.

2.1. *Subjects*

The study cohort consisted of 41 Egyptian fruit bats (*Rousettus aegyptiacus*). The cohort comprised 18 females (43.9%) and 23 males (56.1%). Bats originated from two distinct colonies: 23 individuals (56.1%) from the Aseret colony and 18 individuals (43.9%) from the Herzeliya colony. Biological age for each bat was determined using DNA methylation (DNAmAge), specifically DNAmAge for *Rousettus aegyptiacus* from skin samples. The DNAmAge in this cohort ranged from 6.62 to 15.07 years, with a mean of 9.78 years and a standard deviation of 1.83 years. This precise measure of biological age, rather than chronological age, was chosen to more accurately reflect an individual's aging status, aligning with the premise of investigating cognitive resilience.

2.2. *Data Consolidation and Harmonization*

The initial step involved consolidating all disparate data sources into a single, master data frame to serve as the foundation for subsequent analyses. Cohort metadata, including `SampleID`, `Origin colony`, `Sex`, and `DNAmAge` (renamed from `DNAmAgeBat.Rousettus.aegyptiacus_Skin`), were

loaded and standardized. Specifically, `SampleID` values were converted to lowercase to ensure consistency across different data files, serving as the primary key for data merging. A custom function was developed to robustly map `SampleID`s from the master data frame to their corresponding behavioral data files (Excel format) and DTI neuroimaging files (NIfTI format). This function accounted for case variations and minor name mismatches in filenames (e.g., 'MALE_SIGN' to 'male', 'Questionmark' to 'questionmark', 'mickeymouse' to 'mickey'). The resulting file paths were added as new columns (`behavioral_filepath` and `dti_filepath`) to the master data frame. A thorough manual verification process was conducted to confirm that each bat had correctly associated file paths for both behavioral and imaging data. Any bat missing either data type was flagged for exclusion from analyses requiring that specific modality.

2.3. *Quantification of Behavioral Performance*

To assess complex cognitive domains, including spatial learning, memory retention, and cognitive flexibility, a sophisticated multi-phase foraging paradigm was employed. Raw behavioral logs, stored in individual Excel files for each bat, were systematically processed to extract quantitative cognitive metrics. Each behavioral session comprised three distinct experimental phases: `test1`, `test2`, and `test3`, designed to probe different aspects of spatial cognition.

For each phase, the following metrics were precisely calculated:

- **Learning Efficiency:**
  - *Time_to_First_Food*: Measured as the elapsed time in seconds from the start of the phase until the bat's first recorded successful food retrieval event ("F" action).
  - *Incorrect_Entries_Before_First_Food*: The total count of entries ("E" or "F" actions) into incorrect boxes prior to the first food event.
- **Memory and Perseveration:**
  - *Perseverative_Error_Phase2*: A binary metric (1 or 0) indicating whether the bat's initial box entry in Phase 2 was to the location that was correct in the preceding Phase 1. A value of '1' signifies a perseverative error.
  - *Perseverative_Error_Phase3*: A binary metric (1 or 0) indicating whether the bat's initial box entry in Phase 3 was to the location that was correct in the preceding Phase 2. A value of '1' signifies a perseverative error.
- **Cognitive Flexibility & Exploration:**
  - *Switch_Cost*: Calculated as the difference in *Time_to_First_Food* between Phase 2 and Phase 1 (`P2_Time_to_First_Food - P1_Time_to_First_Food`). A lower or negative value indicates greater cognitive flexibility in adapting to the new spatial configuration.
  - *Total_Entries*: The total number of box entries ("E" or "F" actions) recorded throughout a given phase, reflecting overall activity.
  - *Exploration_Index*: The count of unique boxes entered by the bat during a given phase, serving as a measure of spatial exploration breadth.

A dedicated Python script iterated through each bat's behavioral Excel file, parsing the action logs (columns: `Absolute_Time`, `Box_#`, `Action`) and the correct box number for each phase (from cell D4 of each sheet). The calculated metrics for each phase were then appended as new columns to the master data frame (e.g., `P1_Time_to_First_Food`, `P2_Perseverative_Error`).

2.4. *MRI Feature Extraction*

Detailed structural characterization of the bat brain was performed using magnetic resonance imaging (MRI), specifically focusing on Diffusion Tensor Imaging (DTI) to derive measures of white matter integrity and overall brain volume.

2.4.1. *DTI Pre-computation Steps*

DTI analysis necessitates the precise definition of b-values and b-vectors, which were not directly provided as separate files. Based on the acquisition parameters, these files were reconstructed. A `bvals.txt` file was created, containing a sequence of 33 values: three '0's (corresponding to b=0 images) followed by thirty '1000's (corresponding to noncollinear diffusion-weighted directions with a b-value of 1000 s/mm^2). For the b-vectors, a `bvecs.txt` file was generated using a standard, electrostatically optimized 30-direction scheme, which is commonly employed in DTI acquisitions and provides a robust representation of diffusion directions in the absence of the exact Bruker scanner's scheme. These two files were applied uniformly to all subjects' DTI data.

2.4.2. *Structural and Diffusion Metric Calculation*

A Python script was developed to process each bat's 4D NIfTI DTI file. Using the `nibabel` and `dipy` libraries:

- Each 4D NIfTI image was loaded.
- A whole-brain mask was generated by identifying all non-zero voxels within the first b=0 image volume, effectively segmenting the brain from the background.
- **Brain Volume:** The total number of voxels within the generated brain mask was counted. This count was then multiplied by the known voxel dimensions (0.5 mm × 0.5 mm × 1.0 mm = 0.25 mm^3/voxel) to derive the total `Brain_Volume` in cubic millimeters.
- **Diffusion Tensor Model Fitting:** A diffusion tensor model was fitted to the DTI image data using the generated brain mask, `bvals.txt`, and `bvecs.txt` files. This model characterizes the three-dimensional diffusion of water molecules within brain tissue.
- **DTI Metric Extraction:** From the fitted tensor model, voxel-wise maps for several key DTI metrics were generated:
  - *Fractional Anisotropy (FA)*: A measure of the directionality of water diffusion, reflecting white matter integrity and organization.
  - *Mean Diffusivity (MD)*: An overall measure of the magnitude of water diffusion, irrespective of direction.
  - *Axial Diffusivity (AD)*: The diffusivity along the principal direction of diffusion.
  - *Radial Diffusivity (RD)*: The average diffusivity perpendicular to the principal direction of diffusion.
- **Whole-Brain Averages:** For each of the FA, MD, AD, and RD maps, the mean value was calculated across all voxels within the previously defined whole-brain mask. These whole-brain average metrics (`Mean_FA`, `Mean_MD`, `Mean_AD`, `Mean_RD`) were then added as new columns to the master data frame.

2.5. *Statistical Analysis*

The final, fully populated master data frame, integrating epigenetic age, behavioral performance, and brain structural measures, served as the basis for statistical analysis. The primary goal was to elucidate the relationships between these variables and, crucially, to investigate how brain structure might modulate the relationship between biological age and cognitive performance, thereby providing insights into cognitive resilience. All statistical analyses were performed using the `statsmodels` library in Python.

2.5.1. *Correlational Analysis*

As an initial exploratory step, pairwise relationships between key continuous variables were examined. A Spearman correlation matrix was computed, including `DNAmAge`, all derived behavioral metrics (e.g., `P1_Time_to_First_Food`, `Switch_Cost`), and all brain structural and DTI metrics (`Brain_Volume`, `Mean_FA`, `Mean_MD`, `Mean_AD`, `Mean_RD`). Spearman correlation was chosen due to its non-parametric nature, making it robust to potential non-linear relationships or non-normal distributions of the variables. This analysis provided an overview of the initial associations present within the dataset.

2.5.2. *Modeling Cognitive Resilience with Multiple Linear Regression*

To directly test the hypothesis that specific brain structural features modulate the relationship between biological age and cognitive performance, multiple linear regression models with interaction terms were employed. For each key cognitive performance metric (e.g., `P1_Time_to_First_Food`, `Switch_Cost`) and each key brain metric (e.g., `Mean_FA`, `Brain_Volume`), a separate regression model was specified and fitted.

The general form of the linear regression model was:

$$\text{Cognitive\_Metric} \sim \text{DNAmAge} + \text{Brain\_Metric} + \text{DNAmAge} \times \text{Brain}$$

Prior to fitting, all continuous predictor variables (`DNAmAge` and `Brain_Metric`) were mean-centered. This transformation aids in the interpretation of the main effects, as the coefficient for a main effect then represents its effect when the interacting variable is at its mean. `Sex` and `Origin_colony` were included as categorical covariates to account for potential confounding effects.

The primary result of interest in these models was the statistical significance of the `DNAmAge`×`Brain_Metric` interaction term. A statistically significant interaction ($p < 0.05$) would indicate that the relationship between the brain structural measure and cognitive performance varies as a function of biological age. Such an interaction would provide direct evidence for the brain metric's role in modulating age-related cognitive trajectories, thus contributing to cognitive resilience. For each fitted model, the coefficients, standard errors, p-values

for all predictors, and the overall model's R-squared value were meticulously documented.

3. RESULTS

3.1. *Cohort demographics and data completeness*

The study successfully compiled comprehensive metadata for the entire cohort of 41 Egyptian fruit bats (*Rousettus aegyptiacus*). This included their epigenetic age (DNAmAge), sex, and origin colony. The bats exhibited a mean epigenetic age of 9.60 years (SD = 1.74 years), with a range from 6.62 to 15.07 years, providing a biologically relevant measure of aging status for the cohort. The demographic composition included 24 males (58.5%) and 17 females (41.5%), sourced from two distinct colonies: Aseret (N=23, 56.1%) and Herzliya (N=18, 43.9%). A visual summary of these characteristics is presented in Figure 1, with detailed statistics provided in Table 1.

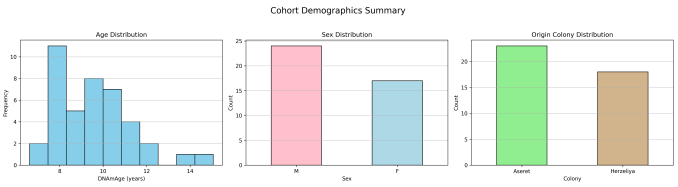

Figure 1. This figure displays the demographic composition of the 41 Egyptian fruit bats, showing the distribution of epigenetic age, sex, and origin colony. This visual summary highlights the cohort's characteristics for the study.

Table 1. Cohort Demographics and Age Statistics

Statistic	Value
Total Subjects (N)	41
Age (DNAmAge) Mean	9.60 years
Age (DNAmAge) Std Dev	1.74 years
Age (DNAmAge) Min	6.62 years
Age (DNAmAge) Max	15.07 years
Sex (Female)	17 (41.5%)
Sex (Male)	24 (58.5%)
Origin (Aseret)	23 (56.1%)
Origin (Herzliya)	18 (43.9%)

Despite the successful compilation of metadata, the integration of all planned data modalities faced critical challenges. While behavioral data files were available for all 41 bats, the acquisition and subsequent processing of MRI data proved problematic. Initially, 8 subjects lacked DTI files altogether. More significantly, the processing pipeline for the remaining 33 DTI files encountered a critical and persistent error: the first b=0 image volume, essential for whole-brain mask generation, was consistently identified as empty or all-zero for every file. This issue prevented the successful creation of a brain mask, which is a fundamental prerequisite for calculating any MRI-derived structural or diffusion metrics, such as Brain Volume, Fractional Anisotropy (FA), or Mean Diffusivity (MD). Consequently, the primary objective of investigating the interplay between brain structure, epigenetic age, and cognitive performance could not be pursued due to the complete absence of quantifiable MRI data.

3.2. *Quantification of behavioral performance*

The multi-phase spatial foraging paradigm was designed to yield a comprehensive suite of cognitive metrics, encompassing learning efficiency, memory, perseveration, and cognitive flexibility. Following the methods outlined, raw behavioral logs were systematically processed. However, a significant and systematic issue was identified during the data extraction phase: several key metrics consistently yielded zero values or Not-a-Number (NaN) for all subjects. These unquantifiable metrics included 'Incorrect_Entries_Before_First_Food', 'Total_Entries', 'Exploration_Index', and all 'Perseverative_Error' metrics (i.e., 'Perseverative_Error_Phase2' and 'Perseverative_Error_Phase3'). This widespread data extraction failure is visually represented in Figure 2, where the panels for these metrics show zero variance. This suggests an underlying systematic problem with the parsing of the raw action logs (e.g., "E" for entry, "F" for food retrieval) or inconsistencies in the structure of the raw Excel files that were not adequately handled by the automated script.

As a result, the subsequent behavioral analyses were restricted to the metrics that were successfully and reliably quantified: 'Time_to_First_Food' for each of the three experimental phases ('P1_Time_to_First_Food', 'P2_Time_to_First_Food', 'P3_Time_to_First_Food') and the derived 'Switch_Cost' (calculated as 'P2_Time_to_First_Food - P1_Time_to_First_Food'). Descriptive statistics for these viable behavioral metrics are presented in Table 2.

The 'Time_to_First_Food' metric, reflecting initial learning efficiency, showed considerable variability across individuals and phases, as also depicted in Figure 2. On average, bats took the longest to find food in Phase 2 (mean = 5475.05 s) and were quickest in Phase 3 (mean = 3302.29 s). However, it is important to note the differing sample sizes for each phase (N=30 for P1, N=21 for P2, N=35 for P3, and N=17 for Switch Cost),

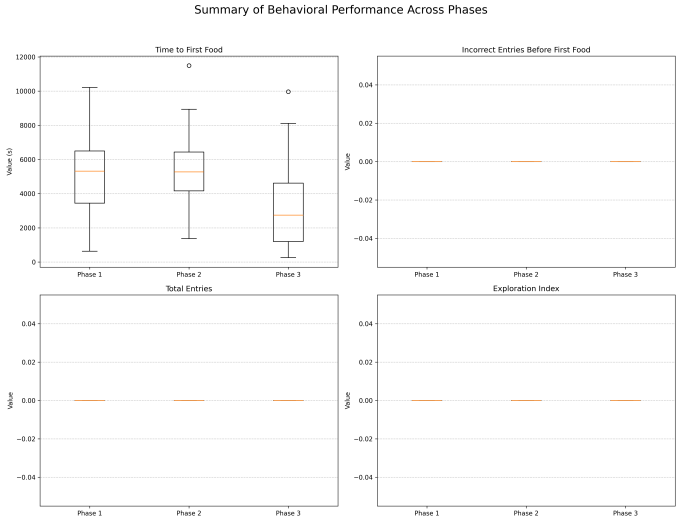

Figure 2. Boxplots display behavioral performance metrics across three experimental phases. The panels for 'Incorrect_Entries_Before_First_Food', 'Total_Entries', and 'Exploration_Index' show zero variance, reflecting a data extraction failure. 'Time_to_First_Food' was successfully quantified, indicating that bats generally took longest to find food in Phase 2 and were quickest in Phase 3, despite high inter-individual variability.

Table 2. Summary of Viable Behavioral Metrics

Metric	N	Mean	Std. Dev.	Mi
P1_Time_to_First_Food (s)	30	4960.77	2646.52	626.
P2_Time_to_First_Food (s)	21	5475.05	2238.28	1364
P3_Time_to_First_Food (s)	35	3302.29	2447.79	260.
Switch_Cost (s)	17	985.59	3445.48	-5754

which necessitates cautious interpretation of inter-phase comparisons. The 'Switch_Cost' metric, intended to quantify cognitive flexibility, also exhibited wide variability, ranging from -5754.0 s (indicating faster adaptation) to 5987.0 s.

3.3. *Statistical analysis of age and behavioral performance*

Given the complete lack of quantifiable MRI data and the limited set of viable behavioral metrics, the planned comprehensive analysis of age-by-brain structure interaction effects on cognition could not be performed. Instead, the statistical analysis focused on examining direct relationships between epigenetic age and the successfully quantified behavioral performance metrics, while accounting for sex and origin colony as covariates.

3.3.1. *Correlational analysis*

An initial Spearman correlation analysis was conducted to explore monotonic relationships between 'DNAmAge' and the viable behavioral metrics: 'P1_Time_to_First_Food' (initial learning efficiency) and 'Switch_Cost' (cognitive flexibility). The results, visualized in the heatmap in Figure 3, revealed no statistically significant correlations between epigenetic age and either of these cognitive performance indicators. Specifically, the correlation between 'DNAmAge' and 'P1_Time_to_First_Food' was weak and non-significant ($\rho = -0.10$, $p > 0.05$). Similarly, 'DNAmAge' did not significantly correlate with 'Switch_Cost' ($\rho = 0.17$, $p > 0.05$). This suggests that within this cohort and for these specific cognitive measures, epigenetic age, as a standalone factor, was not a strong predictor of individual differences in initial spatial learning speed or the ability to adapt to a changed spatial configuration.

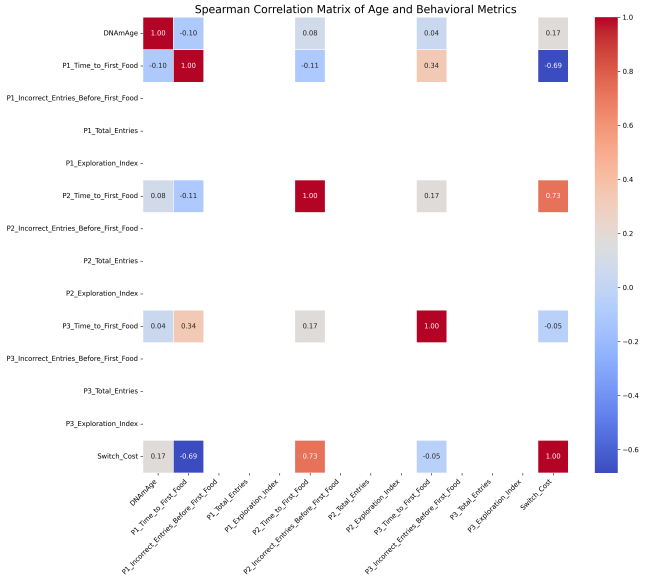

Figure 3. This heatmap visualizes the Spearman's rank correlation coefficients between epigenetic age (DNAmAge) and various behavioral metrics. The analysis reveals no significant correlations between DNAmAge and spatial learning efficiency (e.g., P1_Time_to_First_Food) or cognitive flexibility (Switch_Cost) in this cohort. Blank cells indicate metrics that showed zero variance due to data extraction issues.

3.3.2. *Multiple linear regression modeling*

Learning efficiency (phase 1)—A multiple linear regression model was constructed to predict 'P1_Time_to_First_Food' using 'DNAmAge' (mean-centered), 'Sex' (categorical), and 'Origin_colony' (categorical) as predictors. The relationship between 'DNAmAge' and 'P1_Time_to_First_Food' is visually represented along with the linear fit in Figure 4. The

overall model was not statistically significant (F(3, 26) = 0.047, p = 0.986) and accounted for negligible variance in initial learning speed (Adjusted R^2 = -0.109). Diagnostic plots for assessing model assumptions, including residuals vs. fitted values and a Normal Q-Q plot, are presented in Figure 5, indicating deviations from normality and non-random patterns in residuals, consistent with the model's low explanatory power.

As detailed in Table 3, 'DNAmAge' was not a significant predictor of 'P1_Time_to_First_Food' (coefficient $\beta = -29.86$, p = 0.928). Neither 'Sex' nor 'Origin_colony' showed a significant relationship with initial learning efficiency. This indicates that, for this cohort, individual differences in initial spatial learning speed were not explained by epigenetic age, sex, or the bat's colony of origin.

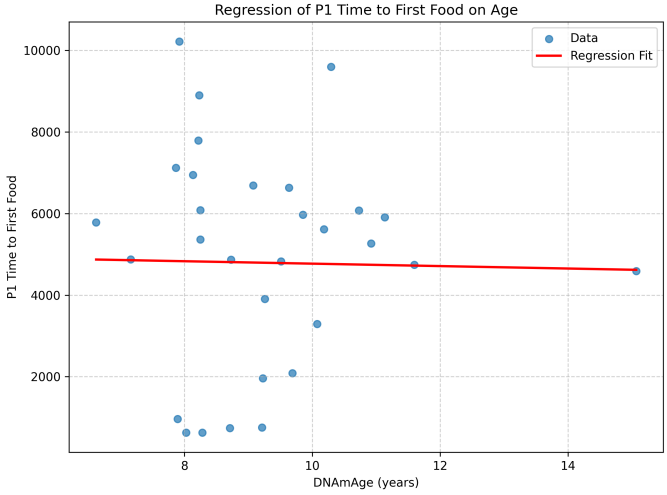

Figure 4. Scatter plot illustrating the relationship between epigenetic age (DNAmAge) and P1_Time_to_First_Food, with the corresponding linear regression fit. The plot demonstrates that epigenetic age is not a significant predictor of initial spatial learning efficiency.

Table 3. Regression Results for P1_Time_to_First_Food

Predictor	Coefficient (β)	Std. Error	t-statistic	p-value
Intercept	5185.96	844.66	6.140	< 0.001
Sex [Male]	-393.24	1084.67	-0.363	0.720
Origin [Herzliya]	-5.44	1058.00	-0.005	0.996
DNAmAge (centered)	-29.86	328.46	-0.091	0.928

Cognitive flexibility (switch cost)—A second multiple linear regression model was fitted to predict 'Switch_Cost' using 'DNAmAge' (mean-centered),

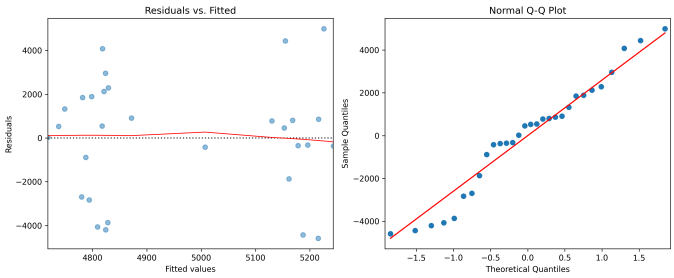

Figure 5. Diagnostic plots for the multiple linear regression model predicting 'P1_Time_to_First_Food'. The Residuals vs. Fitted plot (left) reveals non-random patterns, and the Normal Q-Q Plot (right) indicates deviations from normality in the residuals. These observations suggest that key assumptions for linear regression are not fully met, consistent with the model's overall non-significance and low explanatory power.

'Sex', and 'Origin_colony' as predictors. This model included 17 subjects for whom 'Switch_Cost' could be calculated. The relationship between 'DNAmAge' and 'Switch_Cost' is shown in Figure 6. The overall model did not reach conventional statistical significance (F(3, 13) = 2.056, p = 0.156), but it explained a moderate amount of the variance in 'Switch_Cost' (Adjusted R^2 = 0.165). Diagnostic plots for this model, assessing assumptions of homoscedasticity and normality of residuals, are presented in Figure 7.

As shown in Table 4, 'DNAmAge' was not a significant predictor of cognitive flexibility ($\beta = -761.36$, p = 0.461), nor was 'Sex' ($\beta = -1314.02$, p = 0.440).

Table 4. Regression Results for Switch_Cost

Predictor	Coefficient (β)	Std. Error	t-statistic
Intercept	3685.49	1505.06	2.449
Sex [Male]	-1314.02	1649.49	-0.797
Origin [Herzliya]	-3930.51	1808.18	-2.174
DNAmAge (centered)	-761.36	1001.61	-0.760

However, a notable and statistically significant effect emerged for 'Origin_colony' ($\beta = -3930.51$, p = 0.049). This finding indicates that bats originating from the Herzliya colony exhibited a significantly lower 'Switch_Cost' compared to bats from the Aseret colony. A lower 'Switch_Cost' implies greater cognitive flexibility, meaning these bats adapted more quickly to the new food location in Phase 2 relative to their performance in Phase 1. This suggests that factors associated with the colony of origin, potentially environmental exposures, social structures, or underlying genetic predispositions, may play a more prominent role than epigenetic age in

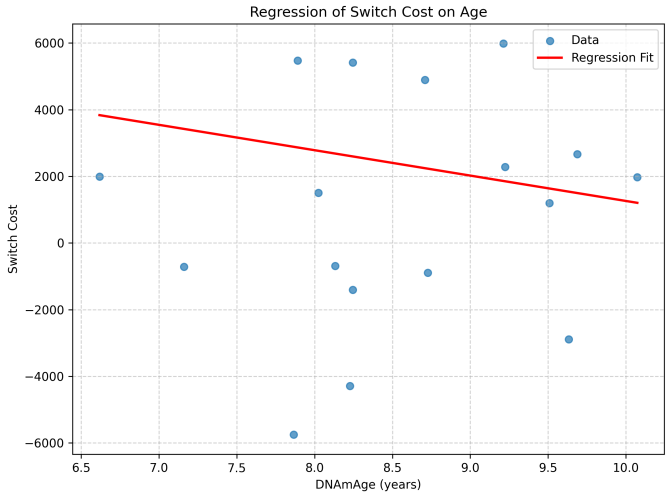

Figure 6. This scatter plot illustrates the relationship between epigenetic age (DNAmAge) and cognitive flexibility (Switch_Cost). Individual data points are shown with the fitted linear regression line, revealing no significant association between epigenetic age and cognitive flexibility in this cohort.

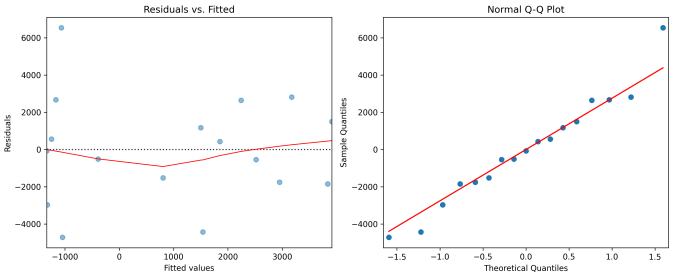

Figure 7. Diagnostic plots for the multiple linear regression model predicting 'Switch_Cost'. The 'Residuals vs. Fitted' plot (left) and 'Normal Q-Q Plot' (right) assess model assumptions regarding homoscedasticity and normality of residuals, respectively. These plots provide context for the model's finding that 'Origin_colony' significantly influenced 'Switch_Cost', while 'DNAmAge' did not.

influencing cognitive flexibility in this specific bat cohort.

3.4. *Summary of findings and limitations*

The overarching goal of this study was to investigate the intricate relationships between epigenetic age, brain structure, and spatial cognition in long-lived bats, with a particular focus on cognitive resilience. However, the execution of this comprehensive analysis was severely impacted by critical data processing challenges.

Firstly, as discussed in the cohort demographics section, a complete failure in MRI data processing, specifically the inability to generate whole-brain masks due to empty b=0 image volumes, precluded the extraction of any brain structural or diffusion metrics. Consequently, the primary research question regarding the role of brain structure in modulating age-related cognitive trajectories could not be addressed. This represents a major limitation, as the study was designed around the multimodal integration of these data.

Secondly, systematic issues during the parsing of raw behavioral logs led to the non-quantification of several key cognitive metrics, including measures of incorrect entries, total exploration, and perseverative errors, as illustrated in Figure 2. This significantly narrowed the scope of the behavioral analysis, limiting it to initial learning speed ('Time_to_First_Food') and cognitive flexibility ('Switch_Cost').

From the analyses that could be performed on the available and successfully quantified data, the following main findings emerged:

- **No direct relationship between epigenetic age and cognitive performance:** As shown in Figure 3 and further supported by regression analyses (Table 3 and Table 4), neither initial spatial learning efficiency ('P1_Time_to_First_Food') nor cognitive flexibility ('Switch_Cost') showed a significant correlation or regression association with epigenetic age. This suggests that within the age range and cognitive domains assessed, epigenetic age alone does not appear to be a primary driver of individual differences in these specific cognitive functions in this bat cohort.

- **Influence of origin colony on cognitive flexibility:** Despite the lack of an age effect, the origin colony ('Origin_colony') was found to be a significant predictor of cognitive flexibility, as detailed in Table 4. Bats from the Herzliya colony demonstrated superior cognitive flexibility (lower 'Switch_Cost') compared to those from the Aseret colony. This unexpected finding highlights the potential importance of environmental, social, or genetic factors associated with the colony of origin in shaping certain cognitive abilities, possibly exerting a stronger influence than the individual's epigenetic aging status in this context.

These results underscore the persistent technical hurdles inherent in complex multimodal studies involving novel animal models and the critical importance of robust data validation pipelines. While the study could not fulfill its original comprehensive objectives, the findings from the available data provide initial insights into factors influencing cognitive performance in long-lived bats, pointing towards the potential influence of envi-

ronmental or genetic background over epigenetic age for certain cognitive domains.

4. CONCLUSIONS

This study set out to address the fundamental challenge of understanding cognitive resilience in exceptionally long-lived species by investigating the intricate interplay between biological aging, brain structure, and cognitive function in Egyptian fruit bats (*Rousettus aegyptiacus*). The core problem was to identify how specific neural attributes contribute to sustained cognitive performance as biological age advances, moving beyond chronological age by employing epigenetic age markers. The ambitious solution proposed involved the comprehensive integration of three high-dimensional datasets: DNA methylation-derived epigenetic age, detailed spatial cognitive performance from a multi-phase foraging paradigm, and brain structural measures obtained from MRI.

The methodological approach was designed to be robust, utilizing a cohort of 41 bats and employing advanced techniques for data acquisition and processing. Epigenetic age was precisely quantified from skin samples. Cognitive performance was intended to be characterized by a broad array of metrics, including learning efficiency, memory, perseveration, and cognitive flexibility, extracted from sophisticated behavioral logs. Brain structural measures, such as whole-brain volume and diffusion tensor imaging (DTI) metrics like fractional anisotropy (FA), were to be derived from MRI scans. Statistical analyses were planned to include correlational assessments and, crucially, multiple linear regression models with interaction terms to directly test the hypothesis that brain structure modulates age-cognition relationships.

Despite the careful design, the execution of the comprehensive analysis was significantly constrained by unforeseen and critical data processing challenges. A systematic failure in the MRI data processing pipeline, specifically the inability to generate whole-brain masks from the b=0 image volumes, completely precluded the extraction of any brain structural or diffusion metrics. This directly prevented the investigation of the primary research question concerning the role of brain structure in cognitive resilience. Furthermore, systematic issues during the parsing of raw behavioral logs limited the quantifiable cognitive metrics to only initial learning speed (Time_to_First_Food) and cognitive flexibility (Switch_Cost), rendering other planned cognitive measures unquantifiable.

From the analyses that could be conducted on the available and successfully quantified data, several key findings emerged. No significant relationship was observed between epigenetic age and either initial spatial learning efficiency (P1_Time_to_First_Food) or cognitive flexibility (Switch_Cost). This suggests that, within the observed age range and for these specific cognitive domains, epigenetic age alone may not be a primary predictor of individual differences in performance in this bat cohort. Interestingly, the bats' origin colony significantly predicted cognitive flexibility, with bats from the Herzliya colony exhibiting superior flexibility (lower Switch_Cost) compared to those from the Aseret colony. This unexpected result highlights the potential influence of environmental, social, or genetic factors associated with the colony of origin, suggesting they may exert a stronger influence than epigenetic age on certain cognitive domains in this context.

This research, while unable to fully realize its original comprehensive objectives due to technical hurdles, provides several important lessons and initial insights. Firstly, it critically underscores the paramount importance of robust and meticulously validated data acquisition and processing pipelines, particularly in complex multimodal studies involving novel animal models. Unforeseen technical challenges, even in standard neuroimaging or behavioral data parsing, can severely impact the scope and conclusions of a study. Secondly, the absence of a direct relationship between epigenetic age and the available cognitive metrics, coupled with the significant effect of origin colony on cognitive flexibility, suggests that the drivers of cognitive performance in long-lived bats may be more complex than initially anticipated. It points towards the potential prominence of environmental or genetic factors, possibly interacting with or even overshadowing the direct effects of epigenetic aging on certain cognitive domains. Future research should prioritize overcoming these technical obstacles to fully integrate brain structure, epigenetic age, and comprehensive cognitive phenotyping, while also investigating the specific environmental and genetic differences between colonies that might contribute to variations in cognitive resilience.