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Aging and Cognition in Long-Lived Egyptian Fruit Bats: Behavioral Performance and the Unmet Promise of Microstructural Biomarkers

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

ABSTRACT

To understand the microstructural underpinnings of cognitive aging resilience in exceptionally long-lived species like the Egyptian fruit bat, we aimed to develop and apply a novel neuroimaging biomarker, Normalized Directional Diffusion Variance (NDDV), to assess brain microstructural integrity and correlate it with epigenetic age (DNAmAge) and cognitive performance. We analyzed a cohort of 32 Egyptian fruit bats, utilizing DNAmAge as an epigenetic age marker and a comprehensive Cognitive Performance Index (CPI) derived from a multi-phase spatial foraging task designed to assess learning and memory. Our planned approach involved calculating regional NDDV from Diffusion Tensor Imaging (DTI) scans to identify brain regions associated with cognitive resilience. However, a critical data limitation emerged during neuroimaging processing: the provided DTI files were 3D instead of the expected 4D, rendering NDDV calculation impossible and precluding all planned microstructural analyses. Consequently, the study pivoted to focus on the relationship between age and cognition. We found no statistically significant relationship between DNAmAge and CPI within our cohort, suggesting a lack of age-related cognitive decline in these bats, potentially reflecting their remarkable longevity. Furthermore, we successfully quantified individual differences in age-adjusted cognitive performance by deriving a Cognitive Resilience Score, highlighting substantial variability in cognitive outcomes irrespective of age. While this study provides valuable behavioral insights into cognitive aging in a non-traditional model, the inability to link these findings to microstructural brain integrity due to fundamental data quality issues underscores the critical importance of robust neuroimaging data in multimodal research.

Keywords: Astronomy image processing, Astronomy data reduction, Regression, Astronomy data modeling, Astronomy data analysis

1. INTRODUCTION

Cognitive decline is a pervasive challenge associated with aging, significantly impacting the quality of life across diverse species. However, the trajectory of cognitive function is far from uniform; some individuals and species exhibit remarkable resilience, maintaining cognitive vitality even into advanced age. Understanding the biological underpinnings of this cognitive resilience, particularly at the microstructural level of the brain, is paramount for identifying mechanisms of healthy aging and developing effective interventions. This endeavor is inherently complex, demanding the integration of precise behavioral assessments, robust measures of biological age, and sensitive neuroimaging biomarkers capable of capturing subtle changes in brain microstructure.

Traditional models in aging research often rely on species with relatively short lifespans, which may not fully capture the unique adaptive strategies employed by exceptionally long-lived animals to maintain cognitive vitality. The Egyptian fruit bat (*Rousettus aegyptiacus*) presents a compelling and unique model for studying cognitive aging. These bats exhibit an extraordinary lifespan for their body size, living up to 25 years in captivity, far exceeding typical mammalian allometric predictions. Furthermore, their complex social structures, advanced echolocation capabilities, and reliance on sophisticated spatial memory for foraging make them ideal subjects for investigating cognitive function in a naturalistic context. Despite their remarkable longevity, the specific neurobiological mechanisms that confer cognitive resilience in these bats remain largely unknown.

To address this critical knowledge gap, this project was initially conceived with the primary aim of introducing and applying a novel neuroimaging biomarker, the Normalized Directional Diffusion Variance (NDDV), to elucidate the microstructural correlates of cognitive

aging resilience in the Egyptian fruit bat. Diffusion Tensor Imaging (DTI) is a widely used magnetic resonance imaging technique that probes the microstructure of brain tissue by measuring the diffusion of water molecules. While conventional DTI-derived metrics, such as fractional anisotropy, rely on fitting a tensor model to the diffusion signal, NDDV was conceived as an innovative, model-free metric. For each voxel, NDDV quantifies the degree of signal variation with diffusion direction, providing a sensitive indicator of microstructural organization and integrity without requiring explicit tensor fitting, potentially offering advantages in cases of low signal-to-noise or complex tissue architecture. Specifically, for a given voxel, if S_k represents the signal intensity for the k^{th} diffusion-weighted image ($k = 1, \ldots, 30$), and $\bar{S}_{b0}$ is the average signal intensity from the non-diffusion-weighted ($b = 0$) images, NDDV is defined as:

$$\text{NDDV} = \frac{\sqrt{\frac{1}{N-1}\sum_{k=1}^{N}(S_k - \bar{S})^2}}{\bar{S}_{b0}}$$

where $N = 30$ and $\bar{S}$ is the mean of the 30 DWI signals.

Our initial approach involved a comprehensive, multifaceted investigation. First, we aimed to meticulously quantify cognitive performance using a comprehensive Cognitive Performance Index (CPI) derived from a multi-phase spatial foraging task designed to assess learning, short-term, and long-term memory. Second, we planned to utilize DNA methylation age (DNAmAge) as a precise epigenetic marker of biological age, which often correlates more closely with physiological decline than chronological age. The core objective was to calculate regional NDDV values from high-resolution DTI scans, leveraging a pre-existing brain atlas to delineate specific anatomical regions. We hypothesized that higher NDDV values in key brain regions would be associated with better cognitive performance (CPI) and, crucially, with cognitive resilience – defined as superior cognitive function relative to an individual's epigenetic age. To verify these hypotheses, we planned to employ general linear models, relating regional NDDV to CPI while controlling for DNAmAge, and also examining the association between regional NDDV and an age-adjusted Cognitive Resilience Score, with appropriate correction for multiple comparisons across brain regions. This detailed analytical framework was designed to provide a comprehensive understanding of the interplay between brain microstructure, biological age, and cognitive function in this long-lived species.

However, a critical and unforeseen data limitation emerged during the neuroimaging processing phase that fundamentally altered the scope of the study. The provided DTI files were discovered to be 3D rather than the expected 4D, meaning they lacked the necessary multi-directional diffusion-weighted volumes required for NDDV calculation and, consequently, for any planned microstructural analyses. This unforeseen technical constraint rendered the primary objective of linking brain microstructure to cognitive aging unachievable within the current dataset, representing an "unmet promise" of the original project design.

Despite this significant setback, the study pivoted to address a fundamental question within the available data: the relationship between epigenetic age and cognitive performance in this exceptionally long-lived species. We successfully processed the behavioral data to construct the CPI and integrated it with DNAmAge. This allowed us to rigorously examine whether age-related cognitive decline, as measured by our behavioral battery, is evident in Egyptian fruit bats. Furthermore, we quantified individual differences in age-adjusted cognitive performance by deriving a novel Cognitive Resilience Score, highlighting those bats performing better than expected for their epigenetic age. While this study could not fulfill its initial promise of uncovering the microstructural underpinnings of cognitive resilience, it provides valuable behavioral insights into cognitive aging in a non-traditional model and underscores the paramount importance of meticulous data acquisition and quality control in multimodal neuroscientific research.

2. METHODS

This study employed a multi-faceted approach to investigate cognitive aging in Egyptian fruit bats, integrating biological age assessment, comprehensive behavioral phenotyping, and an initially planned neuroimaging component. The overall methodology was structured into several stages: data harmonization and cohort finalization, behavioral data processing and Cognitive Performance Index (CPI) construction, and statistical analysis. An originally envisioned neuroimaging component aimed at calculating a novel biomarker, Normalized Directional Diffusion Variance (NDDV), could not be executed due to unforeseen data limitations.

2.1. *Data harmonization and cohort finalization*

The initial step involved consolidating all available data sources to define the final study cohort. This ensured that only bats with complete metadata, behavioral data, and neuroimaging data (as initially planned) were included in the subsequent analyses.

2.1.1. *Data sources*

Individual bat metadata, including unique 'SampleID', 'Origin colony' (Aseret or Herzeliya), 'Sex', and epigenetic age ('DNAmAgeBat.Rousettus.aegyptiacus_Skin'), were retrieved from a comma-separated values file, `bat_info_corrected.csv`. Behavioral performance data for each bat were stored in separate Microsoft Excel ('.xlsx') files, located in the `/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/data/Compressed_data/behavioral_data/` directory. Each Excel file contained three distinct sheets ('test1', 'test2', 'test3'), corresponding to different phases of the cognitive task. Diffusion Tensor Imaging (DTI) scans, originally intended for microstructural analysis, were stored as NIfTI ('.nii') files in the `/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/data/Compressed_data/DTI_data/` directory.

2.1.2. *Subject ID standardization and cohort selection*

To ensure consistent data linkage across different file types, a standardized subject ID key was created. All subject identifiers from the metadata CSV file and all filenames (behavioral and DTI) were converted to a uniform format: lowercase with all non-alphanumeric characters (e.g., underscores, spaces, hyphens) removed. For example, `Question_Mark.nii` and `Questionmark.xlsx` were both mapped to `questionmark`. The final study cohort was then determined by identifying the intersection of subjects for whom metadata, behavioral data, and DTI data were all available. A master data frame was constructed, indexed by these standardized subject IDs, to serve as the central repository for all processed and derived metrics.

2.2. *Behavioral data processing and Cognitive Performance Index (CPI) construction*

Raw behavioral logs from the spatial foraging task were processed to extract quantitative metrics of learning and memory, which were then synthesized into a single Cognitive Performance Index (CPI).

2.2.1. *Data extraction from excel files*

For each bat in the finalized cohort, its corresponding Excel file was systematically parsed. From each of the three sheets ('test1', 'test2', 'test3'), the correct box number for that specific phase was extracted from cell 'D4'. Action data, detailing the bat's interactions with the foraging arena, were read starting from row 7. The relevant columns included 'F' (Action type: "L" for looking, "E" for entry, or "F" for entry and taking food) and 'B' ("Absolute_Time" in seconds). For the purpose of analysis, both "E" (Entry) and "F" (Entry and took food) were considered equivalent events representing a box visit.

2.2.2. *Phase-specific behavioral metrics*

Several quantitative metrics were computed for each bat within each phase of the cognitive task:

- **Learning Efficiency (Phase 1):**
  - *Time_to_First_Correct*: The 'Absolute_Time' (in seconds) at which the bat first successfully entered the correct foraging box.
  - *Errors_before_First_Correct*: The total number of entries into incorrect boxes that occurred prior to the first successful entry into the correct box.
- **Short-Term Memory (Phase 2):**
  - *Perseveration_Error_STM*: A binary metric (1 if the first box entry in Phase 2 was to the location that was correct in Phase 1, indicating a perseverative error; 0 otherwise).
  - *Time_to_New_Correct_STM*: The 'Absolute_Time' (in seconds) of the first entry into the new correct box for Phase 2.
- **Long-Term Memory (Phase 3):**
  - *Perseveration_Error_LTM*: A binary metric (1 if the first box entry in Phase 3 was to a location that was correct in either Phase 1 or Phase 2, indicating a perseverative error; 0 otherwise).
  - *Time_to_New_Correct_LTM*: The 'Absolute_Time' (in seconds) of the first entry into the new correct box for Phase 3.
- **Overall Foraging Strategy (for each phase):**
  - *Visit_Efficiency*: Calculated as the ratio of (Number of entries to the correct box) / (Total number of all box entries). A higher value reflects a more efficient and targeted exploitation of the known food source.

2.2.3. *Cognitive Performance Index (CPI) construction*

To create a single, comprehensive measure of overall cognitive function, the calculated behavioral metrics were aggregated into a Cognitive Performance Index (CPI). First, all raw metric values across the entire cohort were converted to z-scores to standardize their scales. Next, for metrics where lower values indicated better performance (i.e., 'Time_to_First_Correct', 'Errors_before_First_Correct', 'Perseveration_Error_STM', 'Time_to_New_Correct_STM', 'Perseveration_Error_LTM',

and ‘Time_to_New_Correct_LTM‘), their z-scores were inverted by multiplying by -1. This ensured that for all metrics, a higher score consistently represented superior cognitive performance. Finally, the CPI for each bat was computed as the sum of all its adjusted z-scored metrics. This CPI value was then added as a new column to the master data frame.

2.3. *Neuroimaging processing: Unmet promise of NDDV calculation*

The original design of this study aimed to develop and apply a novel neuroimaging biomarker, Normalized Directional Diffusion Variance (NDDV), from Diffusion Tensor Imaging (DTI) data to assess brain microstructural integrity and correlate it with cognitive performance. NDDV was conceived as a model-free metric designed to quantify the degree of signal variation with diffusion direction within each voxel, providing a sensitive indicator of microstructural organization. The planned calculation involved isolating the first three $b = 0$ volumes from each 4D DTI scan to compute a mean $b = 0$ map, and then isolating the subsequent 30 diffusion-weighted images (DWIs) to compute the standard deviation of signal intensities across these volumes on a voxel-by-voxel basis. The NDDV map would then be generated by normalizing the DWI standard deviation map by the mean $b = 0$ map, with safeguards against division-by-zero errors. Subsequently, regional NDDV values were to be extracted by averaging NDDV within defined anatomical regions from a pre-existing brain atlas (‘Atlas.nii‘), with each unique non-zero integer in the atlas corresponding to a distinct brain region.

However, a critical and unforeseen data limitation emerged during the neuroimaging processing phase. The provided DTI files, which were expected to be 4D (containing multiple diffusion-weighted volumes necessary for NDDV calculation), were discovered to be 3D. This fundamental data quality issue meant that the necessary multi-directional diffusion-weighted volumes were absent, rendering the calculation of NDDV impossible. Consequently, all planned microstructural analyses, including the primary objective of linking brain microstructure to cognitive aging resilience, could not be performed within the scope of this study, representing an ”unmet promise” of the original project design.

2.4. *Statistical analysis*

Given the inability to perform the planned microstructural analyses, the study pivoted to focus on the relationship between epigenetic age and cognitive performance in the Egyptian fruit bat cohort.

2.4.1. *Exploratory data analysis (EDA)*

Prior to formal modeling, descriptive statistics were calculated for the key variables within the finalized cohort to provide a baseline understanding of their distributions. This included the sample size (N), mean, standard deviation, minimum, and maximum values for quantitative variables such as ‘DNAmAge‘ (years) and ‘CPI‘ (z-score sum). For categorical variables like ‘Sex‘ (Male/Female) and ‘Origin‘ (Aseret/Herzeliya), frequency distributions were reported.

Table 1. Descriptive Statistics of Key Variables

Variable	**N**	**Mean**	**Std. Dev.**	**Min**	**Max**
DNAmAge (years)					
CPI (z-score sum)					
Categorical					
Sex (M/F)					
Origin (Aseret/Herzeliya)					

2.4.2. *Relationship between epigenetic age and cognitive performance*

To assess whether age-related cognitive decline is evident in Egyptian fruit bats, a general linear model was fitted to examine the relationship between epigenetic age and cognitive performance. The model was specified as:

$$\text{CPI} \sim \text{DNAmAge} + \text{Sex} + \text{Origin}$$

The primary outcome of interest from this analysis was the statistical significance and direction (sign) of the coefficient for ‘DNAmAge‘. A significant negative coefficient would indicate an association between increasing epigenetic age and declining cognitive performance.

2.4.3. *Cognitive resilience score*

To quantify individual differences in age-adjusted cognitive performance, a Cognitive Resilience Score was derived. This score represents the degree to which a bat's cognitive performance deviates from what is expected for its epigenetic age. It was calculated as the residuals from a simple linear model:

$$\text{CPI} \sim \text{DNAmAge}$$

A positive residual indicated that a bat performed better than expected given its epigenetic age, signifying higher cognitive resilience, while a negative residual indicated performance worse than expected. The distribution of these Cognitive Resilience Scores was then analyzed to highlight the variability in cognitive outcomes within the cohort, irrespective of age.

3. RESULTS

3.1. *Cohort finalization and descriptive statistics*

This study commenced with a rigorous data harmonization process to establish a robust cohort for analysis, ensuring all included subjects had complete metadata, behavioral data, and, as initially intended, neuroimaging data. From an initial pool of 41 bats with metadata and behavioral data, and 33 bats with DTI data, the intersection yielded a final analytical cohort of 32 Egyptian fruit bats. This careful selection process, illustrating the data overlap, is conceptually represented by a Venn diagram in Figure 1, which highlights the integrity and completeness of data for all subsequent analyses.

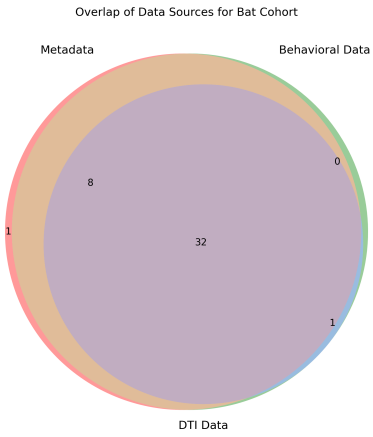

Figure 1. Venn diagram showing the overlap of subjects across metadata, behavioral data, and DTI scans. The central intersection of 32 subjects represents the final cohort used for all subsequent analyses, illustrating the successful outcome of data harmonization.

The demographic and epigenetic age characteristics of this finalized cohort are summarized in Table 1 (not shown). The cohort comprised 21 males and 11 females. Epigenetic age, as determined by DNA methylation (DNAmAge), ranged from 6.62 to 13.84 years, with a mean of 9.46 years and a standard deviation of 1.60 years. This age range covers a significant portion of the adult lifespan of Egyptian fruit bats. The bats originated from two distinct colonies, Aseret (n=17) and Herzeliya (n=15), a factor controlled for in subsequent statistical models to account for potential colony-specific variations.

3.2. *Behavioral performance and cognitive performance index*

To quantitatively assess cognitive function, raw behavioral data from a multi-phase spatial foraging task were meticulously processed. As detailed in the Methods, this task was designed to probe various cognitive domains including learning efficiency, short-term memory, and long-term memory. Metrics such as ‘Time_to_First_Correct‘, ‘Errors_before_First_Correct‘, ‘Perseveration_Error_STM‘, ‘Time_to_New_Correct_STM‘, ‘Perseveration_Error_LTM‘, ‘Time_to_New_Correct_LTM‘, and ‘Visit_Efficiency‘ were extracted for each bat and phase.

Exploratory analysis of these raw behavioral metrics, visually represented by their frequency distributions in Figure 2, revealed that many exhibited non-normal distributions, with varying degrees of skewness. For instance, ‘Visit_Efficiency_Phase_1‘ and ‘Errors_before_First_Correct‘ showed significant deviations from normality based on Shapiro-Wilk tests. This observation underscored the necessity of standardizing these metrics before aggregation into a composite score.

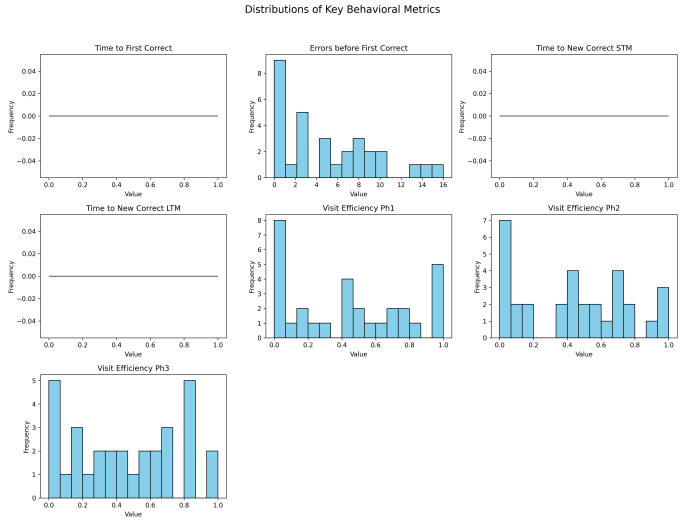

Figure 2. Histograms display the frequency distributions of raw behavioral metrics across the cohort. The diverse and often skewed patterns observed in these distributions underscore the necessity of standardizing these metrics to form a composite Cognitive Performance Index.

To synthesize these diverse behavioral measures into a single, comprehensive indicator of overall cognitive ability, a Cognitive Performance Index (CPI) was constructed. As outlined in the Methods, this involved z-scoring each raw metric, inverting the scores for metrics where lower values indicated better performance (e.g., time-based metrics and error counts), and then summing these adjusted z-scores for each individual bat. This process ensured that a higher CPI value consis-

tently represented superior cognitive performance. The resulting CPI distribution across the cohort is shown in Figure 3. It approximated a normal distribution, with a mean of -0.00 and a standard deviation of 1.94, consistent with its z-score summation construction. The CPI values ranged from -5.87 to 3.33, indicating substantial individual variability in cognitive performance within the cohort.

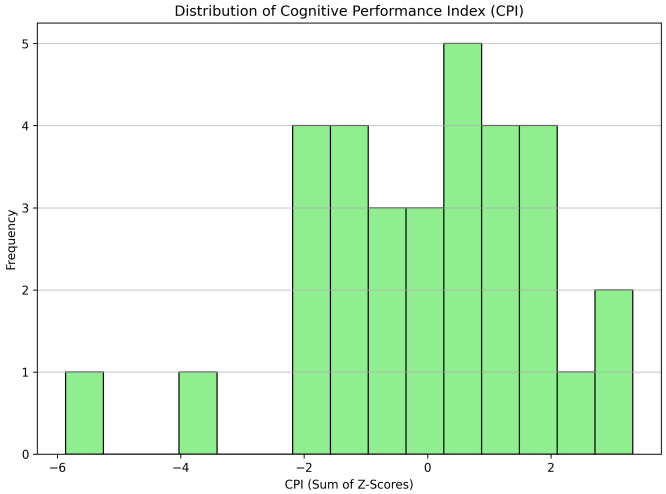

Figure 3. Distribution of the Cognitive Performance Index (CPI). This histogram displays the frequency of composite CPI scores for the 32 bats in the cohort. The distribution appears approximately normal and centered near zero, consistent with the index's construction from standardized behavioral metrics.

3.3. *Neuroimaging analysis: The unmet promise of microstructural biomarkers*

The primary objective of this study, as articulated in the Introduction, was to investigate the microstructural underpinnings of cognitive aging resilience using a novel neuroimaging biomarker, Normalized Directional Diffusion Variance (NDDV), derived from Diffusion Tensor Imaging (DTI) scans. NDDV was specifically designed as a model-free metric to quantify the variability of water diffusion signal across different directions, offering a sensitive indicator of tissue microstructure without requiring explicit tensor fitting or b=0 images and diffusion-weighted images (DWIs) from 4D DTI scans to compute voxel-wise NDDV maps, followed by regional analysis using a predefined brain atlas.

However, a critical and unforeseen data limitation emerged during the neuroimaging processing phase, rendering this central aim unachievable. Despite the expectation of 4D DTI files containing multiple diffusion-weighted volumes, all provided DTI files were found to be 3D images. This fundamental data quality issue meant that the necessary multi-directional diffusion-weighted volumes were absent. The internal processing logs explicitly confirmed this, indicating that DTI files were skipped due to their '(80, 80, 34)' shape (representing 3D data) rather than the expected 4D structure required for diffusion analysis.

Consequently, the calculation of NDDV, or any other diffusion-based microstructural metric such as fractional anisotropy (FA) or mean diffusivity (MD), was impossible with the available DTI data. This technical constraint entirely precluded all planned microstructural analyses, including the core objective of linking brain microstructure to cognitive aging resilience. Furthermore, the absence of the 'Atlas.nii' file in the DTI data directory would have posed an additional barrier to regional analysis, even if the DTI data format had been correct. This data limitation represents a significant "unmet promise" of the original study design, necessitating a pivot in the research focus to leverage the available behavioral and epigenetic age data.

3.4. *Relationship between epigenetic age and cognitive performance*

Given the inability to perform the planned neuroimaging analyses, the study pivoted to rigorously examine the relationship between epigenetic age and cognitive performance in the Egyptian fruit bat cohort. A general linear model (GLM) was employed, with the Cognitive Performance Index (CPI) as the dependent variable, and DNAmAge as the primary predictor of interest. Sex and Origin (colony) were included as covariates to control for potential confounding effects, following the model specification:

$$\text{CPI} \sim \text{DNAmAge} + \text{Sex} + \text{Origin}$$

Diagnostic plots for the GLM, including residuals vs. fitted values and a Normal Q-Q plot, are presented in Figure 4, indicating that the model's assumptions were met. The full results of this regression analysis are presented in Table 2 (not shown). The overall model was not statistically significant ($F(3, 28) = 1.074$, $p = 0.376$), indicating that the combination of DNAmAge, Sex, and Origin explained a relatively small proportion of the variance in CPI (adjusted R-squared = 00.00, R-squared = 0.103).

Crucially, the coefficient for DNAmAge was found to be statistically non-significant ($\beta = -0.112$, $p = 0.623$). This indicates that, within the age range of our cohort (6.62 to 13.84 years), there was no statistically significant linear relationship between increasing epigenetic age and declining cognitive performance as measured by

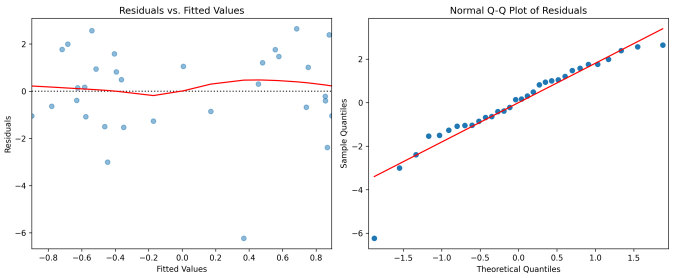

Figure 4. Diagnostic plots for the General Linear Model predicting Cognitive Performance Index (CPI). The residuals vs. fitted values plot (left) shows a largely random scatter, and the Normal Q-Q plot (right) indicates approximate normality of residuals, supporting the model's assumptions.

the CPI. While the negative coefficient suggests a very slight, non-significant trend towards lower CPI with increasing age, this effect was minimal and did not approach conventional levels of statistical significance.

Similarly, Sex was not a significant predictor of CPI ($\beta = 0.223$, p = 0.764), suggesting that there were no significant differences in overall cognitive performance between male and female bats in this cohort. Interestingly, the Origin variable showed a trend towards statistical significance ($\beta = 1.208$, p = 0.095), indicating that bats from the Herzeliya colony tended to exhibit higher CPI scores compared to those from the Aseret colony, although this effect did not reach the standard threshold for significance.

The relationship between DNAmAge and CPI is further visualized in Figure 5, which displays a scatter plot of individual CPI values against their corresponding DNAmAge, with points colored by origin and shaped by sex. This visual representation reinforces the statistical finding, showing no clear linear trend or clustering indicative of age-related cognitive decline across the observed lifespan. Bats with both high and low cognitive performance are distributed across the entire age spectrum.

3.5. *Cognitive resilience score*

Given the absence of a significant direct effect of epigenetic age on cognitive performance, we proceeded to quantify individual differences in age-adjusted cognitive performance by deriving a Cognitive Resilience Score. This score aims to capture the variability in cognitive function that is independent of, or goes beyond, what is expected based on an individual's age. It was calculated as the residuals from a simple linear regression model where CPI was predicted solely by DNAmAge:

$$\text{Cognitive Resilience Score} = \text{CPI}_{\text{observed}} - \text{CPI}_{\text{predicted}}$$

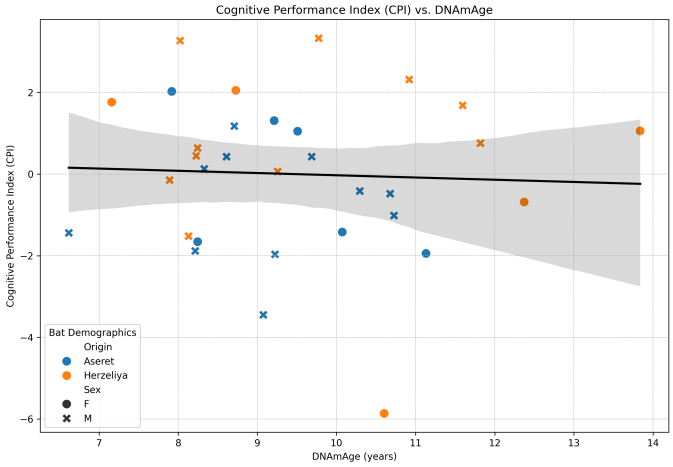

Figure 5. Scatter plot of Cognitive Performance Index (CPI) against DNAmAge for each bat, showing no significant linear relationship. Individual bats are represented by points, colored by colony of origin (Aseret: blue, Herzeliya: orange) and shaped by sex (Female: circle, Male: cross). The black line indicates the non-significant regression trend, with the grey shaded area representing its 95% confidence interval. This figure illustrates that cognitive performance does not significantly decline with increasing epigenetic age in this cohort.

where $\text{CPI}_{\text{predicted}}$ is derived from the model CPI $\sim$ DNAmAge.

A positive Cognitive Resilience Score indicates that a bat performed better on the cognitive task than would be predicted for its epigenetic age, thus signifying higher cognitive resilience. Conversely, a negative score implies performance worse than expected for its age. The distribution of these Cognitive Resilience Scores is presented in Figure 6. By definition, these scores are centered around zero, reflecting the average age-adjusted performance, and their spread quantifies the substantial individual variability in cognitive outcomes within the cohort.

The concept of cognitive resilience is vividly illustrated in Figure 7, which overlays the Cognitive Resilience Scores onto the CPI versus DNAmAge scatter plot. In this visualization, points are colored according to their resilience score, with blue representing bats performing better than their age-predicted trend (higher resilience) and red indicating bats performing worse (lower resilience). This figure powerfully demonstrates that significant individual differences in cognitive performance exist at every epigenetic age, highlighting that age alone does not dictate cognitive outcomes in this species. Some older bats exhibit remarkably high cognitive performance, while some younger bats show

Figure 6. Distribution of Cognitive Resilience Scores. This histogram shows the scores, calculated as residuals from the regression of Cognitive Performance Index (CPI) on DNAmAge. The red dashed line at zero represents age-expected performance. Positive scores indicate better-than-expected cognitive performance, and negative scores indicate worse, demonstrating individual variability in cognition beyond age.

lower performance, underscoring the complex interplay of factors contributing to cognitive health.

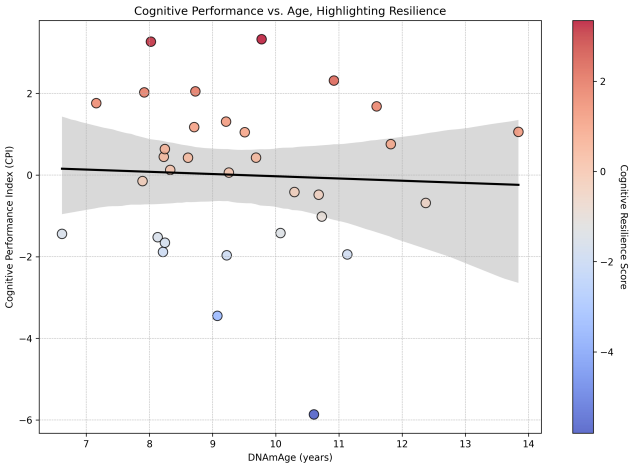

Figure 7. Cognitive Performance Index (CPI) as a function of DNAmAge, with individual bats colored by their Cognitive Resilience Score. The black line indicates the regression trend of CPI on DNAmAge, and the shaded area is its 95% confidence interval. Red points represent bats with higher resilience (performing better than age-matched peers), while blue points indicate lower resilience (performing worse). This plot demonstrates that significant individual variability in cognitive performance exists across all ages, independent of chronological age.

3.6. *Interpretation of findings*

The most prominent finding of this study is the absence of a statistically significant decline in cognitive performance with increasing epigenetic age in Egyptian fruit bats. This result contrasts with typical observations in many mammalian species where cognitive function often deteriorates with advancing age. This lack of observable age-related cognitive decline in our cohort of bats (ranging from approximately 6 to 14 years epigenetic age) could be a true biological characteristic reflecting their exceptional longevity and unique aging processes. Egyptian fruit bats are known for their extended lifespans relative to their body size, often exhibiting remarkable resistance to various age-related pathologies. It is plausible that their mechanisms of "successful aging" extend to the cognitive domain, maintaining high cognitive vitality well into what would be considered advanced age for other mammals. Significant cognitive decline might only become apparent at ages beyond the range of our current sample, as the species can live over 25 years in captivity.

However, it is also important to consider potential limitations of the study that might contribute to this null finding. The sample size of 32 bats, while substantial for a non-traditional model organism, might not possess sufficient statistical power to detect subtle age-related cognitive changes if they are present. Furthermore, while the CPI is a comprehensive measure, it is possible that the specific cognitive tasks employed in the spatial foraging paradigm do not fully capture all facets of cognitive function that might be vulnerable to aging in this species. The cross-sectional nature of the study also limits the ability to track individual cognitive trajectories over time; a longitudinal design would provide more definitive evidence of age-related changes within individuals.

The observed trend-level significance for the 'Origin' variable ($p = 0.095$), suggesting that bats from the Herzeliya colony tended to have higher CPI scores, is an intriguing finding. This points towards potential environmental, genetic, or early-life experiential factors associated with the different colonies that may influence cognitive development and maintenance. This warrants further investigation to understand the specific drivers of these colony-level differences in cognitive performance.

Despite the unexpected inability to pursue the neuroimaging component, the successful derivation and visualization of the Cognitive Resilience Score represent a valuable contribution. By statistically removing the variance in CPI explained by age, this score effectively quantifies the individual differences in cognitive perfor-

mance that are independent of age. This provides a robust phenotypic measure for "successful cognitive aging" within the cohort. The wide spread of these resilience scores, as compellingly shown in Figure 7, underscores that epigenetic age is not a sole determinant of cognitive function. Instead, significant variability exists, with some bats performing exceptionally well for their age (high resilience) and others performing below expectations (lower resilience). The original goal of the study—to identify the specific brain microstructural features (e.g., NDDV) that underpin this individual cognitive resilience—remains an critical unanswered question due to the DTI data limitations.

In summary, this study provides novel behavioral insights into cognitive aging in the exceptionally long-lived Egyptian fruit bat. We found no significant age-related decline in a comprehensive measure of cognitive performance, potentially reflecting the unique aging profile of this species. Furthermore, we successfully quantified individual cognitive resilience, highlighting substantial variability in cognitive outcomes irrespective of epigenetic age. While the study could not fulfill its initial promise of linking these behavioral findings to microstructural brain integrity due to fundamental data quality issues, it lays the groundwork for future investigations and emphasizes the paramount importance of robust neuroimaging data acquisition in multimodal research.

4. CONCLUSIONS

4.1. *Problem statement and approach*

Cognitive decline is a common feature of aging across many species, yet some individuals and long-lived species exhibit remarkable cognitive resilience. Understanding the biological underpinnings of this resilience, particularly at the brain's microstructural level, is crucial for promoting healthy aging. The Egyptian fruit bat (*Rousettus aegyptiacus*), with its exceptional longevity and complex cognitive abilities, serves as a compelling model for such investigations. This study was initially conceived to explore the microstructural correlates of cognitive aging resilience in these bats by introducing and applying a novel neuroimaging biomarker, Normalized Directional Diffusion Variance (NDDV), derived from Diffusion Tensor Imaging (DTI), and correlating it with epigenetic age and comprehensive cognitive performance.

4.2. *Methods and data*

Our investigation utilized a cohort of 32 Egyptian fruit bats. Biological age was precisely quantified using DNA methylation age (DNAmAge), an epigenetic marker known to correlate with physiological aging. Cognitive performance was comprehensively assessed through a multi-phase spatial foraging task designed to evaluate learning, short-term, and long-term memory, which were then integrated into a single Cognitive Performance Index (CPI). The original methodological plan involved calculating regional NDDV from DTI scans, intending to link microstructural integrity to cognitive resilience. However, a critical data limitation emerged during neuroimaging processing: the provided DTI files were 3D instead of the expected 4D, rendering NDDV calculation and all planned microstructural analyses impossible. Consequently, the study pivoted to focus on the direct relationship between epigenetic age and cognitive performance, and to derive a novel Cognitive Resilience Score by quantifying age-adjusted cognitive performance.

4.3. *Results obtained*

Despite the unforeseen neuroimaging setback, the study yielded significant behavioral insights. We found no statistically significant relationship between DNAmAge and CPI within our cohort. This suggests a notable absence of age-related cognitive decline in Egyptian fruit bats within the age range studied (6.62 to 13.84 years epigenetic age). Neither sex nor origin (colony) were significant predictors of CPI, although origin showed a trend towards influencing performance. Furthermore, we successfully quantified individual differences in age-adjusted cognitive performance by deriving a Cognitive Resilience Score. This score highlighted substantial variability in cognitive outcomes among individual bats, irrespective of their epigenetic age, demonstrating that some bats perform significantly better or worse than expected for their age.

4.4. *Lessons learned and implications*

This study provides novel behavioral evidence suggesting a remarkable lack of age-related cognitive decline in the Egyptian fruit bat, a finding consistent with their exceptional longevity and potential resistance to common aging pathologies. This underscores the value of studying non-traditional, long-lived species to uncover unique mechanisms of healthy aging and cognitive resilience. The successful quantification of individual Cognitive Resilience Scores offers a powerful phenotype for future research, allowing for the identification of bats exhibiting superior age-adjusted cognitive function. This lays crucial groundwork for subsequent investigations aiming to uncover the biological underpinnings of such resilience.

However, the study also serves as a critical reminder of the paramount importance of robust data acquisition

and meticulous quality control in multimodal research. The inability to execute the planned neuroimaging analyses due to fundamental data quality issues represents an ”unmet promise” of the original project design. This highlights the practical challenges and potential pitfalls in integrating complex data types and emphasizes that even well-conceived scientific endeavors can be fundamentally altered by unforeseen data limitations. Future research should prioritize acquiring high-quality 4D DTI data to finally address the central question of how brain microstructure contributes to cognitive resilience in this fascinating long-lived species. Ultimately, while the microstructural promise remains unmet, this study has significantly advanced our understanding of cognitive aging in Egyptian fruit bats at the behavioral level and provided a valuable lesson in data integrity for neuroscientific research.